The Drosophila Par domain protein I gene, Pdp1, is a regulator of larval growth, mitosis and endoreplication

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Abstract

PDP1 is a basic leucine zipper (bZip) transcription factor that is expressed at high levels in the muscle, epidermis, gut and fat body of the developing Drosophila embryo. We have identified three mutant alleles of Pdp1, each having a similar phenotype. Here, we describe in detail the Pdp1 mutant allele, Pdp1p205, which is null for both Pdp1 RNA and protein. Interestingly, homozygous Pdp1p205 embryos develop normally, hatch and become viable larvae. Analyses of Pdp1 null mutant embryos reveal that the overall muscle pattern is normal as is the patterning of the gut and fat body. Pdp1p205 larvae also appear to have normal muscle and gut function and respond to ecdysone. These larvae, however, are severely growth delayed and arrested. Furthermore, although Pdp1 null larvae live a normal life span, they do not form pupae and thus do not give rise to eclosed flies. The stunted growth of Pdp1p205 larvae is accompanied by defects in mitosis and endoreplication similar to that associated with nutritional deprivation. The cellular defects resulting from the Pdp1p205 mutation are not cell autonomous. Moreover, PDP1 expression is sensitive to nutritional conditions, suggesting a link between nutrition, PDP1 isotype expression and growth. These results indicate that Pdp1 has a critical role in coordinating growth and DNA replication.

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Introduction

Drosophila melanogaster has four distinct life stages: the embryo, larva, pupa and adult. During embryogenesis, the organ systems for the larva are developed, and the anlagen for the adult organ systems are specified. While the organ systems develop during embryogenesis, the purpose of the larval stage is mostly growth. The larva increases its mass 200-fold in the course of 4 days primarily by an increase in cell size, rather than by an increase in cell number (reviewed in Edgar and Orr-Weaver, 2001). The growth of the larva is stimulated by food intake and involves modified mitotic cycles, the so-called endocycles, in which the DNA is replicated, but the cells do not divide. This leads to a large polyploidization of the larval cells ranging from 16C up to 1024C. These polyploid cells are much larger than diploid cells and are the major contributor to larval growth (Edgar et al., 2001). The endoreplication of most larval tissue parallels growth. Experimental inhibition of the endocycles via the use of DNA replication inhibitors, mutations in genes essential for DNA replication or overexpression of S-phase inhibitors, shows that the increase in ploidy is essential for both cell and larval growth (Edgar and Orr-Weaver, 2001). In addition to these endocycles, certain cells that will contribute to the adult body, the imaginal cells, undergo mitoses. These presumptive adult tissues include the imaginal discs, larval neuroblasts, the mitotic islands in the gut and the abdominal histoblast nests.

The regulation of larval endocycles is particularly sensitive to nutritional conditions, especially dietary intake of amino acids (Britton and Edgar, 1998). Under conditions that arrest growth (starvation), the endocycles are halted. In addition, recent evidence suggests the existence of a cell-type-specific oscillator that is sensitive to nutritional conditions and metabolic rate (Edgar et al., 2001). By altering the nutritional conditions, the larval endocycles can be stopped, restarted, slowed and accelerated. In addition, mutations in dTOR (Drosophila Target of Rapamycin), a kinase that is thought
to link nutritional conditions (amino acid intake) with the translation machinery and protein synthesis, have endoreplication defects (Zhang et al., 2000; Jacinto and Hall, 2003; Neufeld, 2004; Schmelze and Hall, 2000). Other mutations that have defective protein synthesis or dietary uptake of amino acids are also growth and endoreplication defective (Galloni and Edgar, 1999; Lachance et al., 2002).

This link to nutritional intake and S-phase progression is different in the mitotic cells of the larva. For example, one group of cells that begin to proliferate after hatching is the larval neuroblast cells. While these cells are dependent on the intake of dietary amino acids to initiate post-hatching proliferation, they continue to proliferate for several days after food withdrawal. This is in contrast to endoreplicating cells which cease DNA replication within 24 h of food withdrawal. The regulation of mitosis in the brain and imaginal tissues appears to be regulated at least in part by a diffusible factor(s) from the larval fat body (Kawamura et al., 1999; Martin et al., 2000; Bryant, 2001).

Recent studies have also shown the importance of the insulin-signaling pathway on growth, endoreplication and mitosis in the larva. Mutations in the insulin receptor (InR) or components of the growth factor-signaling pathways, such as Chico, Dp110, Dakt, PI3K, and dTOR lead to either decreased body size, larval growth delays and/or larval lethality due to decreased cell size (ploidy), decreased cell number (mitosis) or both (Britton et al., 2002; Bryant, 2001; Garofalo, 2002; Hafen, 2004; Oldham et al., 2002; Weinkove et al., 1999). All of these results point to a tight interplay between the nutritional intake of amino acids, S-phase progression and growth. The rapid and massive amount of growth on the part of the larva requires that the cuticle of the developing larva be shed. This molting, or ecdysis, is controlled by the hormone ecdysone and its coordinate receptor (Henrich et al., 1993). Thus, larval development is coordinated by growth in response to nutrition (and other environmental factors) and hormonal signals.

In this paper, we describe the growth delay/arrest phenotype of a null mutation in the transcription factor PAR domain protein 1 (PDP1). This factor was originally isolated in a screen for regulators of Tropomyosin I gene expression in embryonic muscle (Lin et al., 1997). PDP1 is a basic leucine zipper (bZIP) transcription factor that shares high homology with several vertebrate proteins including D-Box binding protein (DBP) from mouse, hepatic leukemia factor (HLF) from human, vitellogenin binding protein (VBP) from chicken and thyrotrrophic embryonic factor (TEF) from zebrafish (Burch and Davis, 1994; Hunger et al., 1992; Narayanan et al., 1998). These proteins make up a subfamily of bZIP transcription factors called the PAR bZIP family. Pdp1 differentially expresses several isoforms in a variety of tissues in the embryo and larva, including the gut, body wall muscle pharyngeal muscle, malpighian tubules, hindgut, fat body and brain (Reddy et al., 2000). Moreover, expression of Pdp1 in the brain has been shown to be a key component in a double feedback loop regulating the circadian clock (Cyran et al., 2003; Allada, 2003).

Our findings reported here indicate that despite the widespread expression of PDP1, Pdp1<sup>l(205)</sup> null mutation embryos are viable. The Pdp1<sup>l(205)</sup> larvae, however, are severely growth delayed or arrested, depending on the environmental conditions, and can survive for long periods of time (up to 30 days) as larvae. Also, as reported previously, these larvae are arrhythmic due to the involvement of PDP1 in regulating circadian rhythm (Cyran et al., 2003). The growth-retarded phenotype appears to be due to defects in mitosis and endoreplication in the larva which are not cell autonomous and can be ameliorated by feeding a high sucrose diet that allows them to form pupae but not viable adults. Pdp1 function thus appears to be necessary for proper growth through its involvement in endoreplication and mitosis. Our studies also show that the expression of PDP1 is sensitive to nutritional conditions, suggesting that PDP1 may serve as a transcriptional link between nutritional conditions in the environment and growth of the organism.

**Methods**

**Pdp1<sup>l(205)</sup> mutant and other fly stocks**

To generate deletions that disrupt the Pdp1 locus, a P-element mobilization screen was employed. The P-element mobilized for this screen, p[lacW]<sup>l(3)s071411</sup>, was obtained from the Szeged, Hungary, *Drosophila melanogaster* P Insertion Mutant Stock Center. This line has a P [lacW] insertion approximately 4 kb downstream of the Pdp1 gene. Although this P-element was originally designated as pupal lethal, the lethality was determined to be due to mutation(s) outside of this insertion site. Homozygous viable and fertile flies were generated which carry the Pdp1<sup>l(205)</sup> P-element insertion. To generate the Pdp1<sup>l(205)</sup> mutation, w; P<l(3)s071411> flies were crossed to w;Sh<sup>A2–3</sup>/TM6 flies. The resulting w;Sh<sup>A2–3</sup>/07411 male progeny were crossed to w;TM35B/TM6 females. These lines were then crossed to Df(3L)HnD-1/TM6, Sh (from Kalpana White, Brandeis University), which removes Hn through pbl and includes Pdp1.

To generate small mutations in the Pdp1 gene, 4-day-old *ru h st ry e* male flies were treated with EMS. These male flies were then mated individually to Df(3L)HnD-1/TM6b, Tb female virgins. Single *ru h st ry e* male/TM6b males were backcrossed to (3L)HnD-1/TM6b females, and the resulting offspring were scored for viability over the deficiency. Lethal offspring were recovered over the Tub6, Tb balancer. Five complementation groups were uncovered in this screen. Two of the complementation groups isolated in this screen were allelic to pbl and syd. One of the other complementation groups, containing two alleles, Pdp1<sup>p205</sup> and Pdp1<sup>q205</sup>, was shown to represent mutations in the Pdp1 gene.

**Inverse PCR and plasmid rescue**

The molecular basis for the Pdp1<sup>l(205)</sup> mutation was determined by inverse PCR and plasmid rescue (Ochman et al., 1988). Thirty anesthetized flies, either *P<l(3)s071411>* or Pdp1<sup>p205</sup> were collected in microfuge tubes and frozen at −80°C. These flies were lysed, and the DNA was isopropanol precipitated according to standard genomic DNA isolation protocols (Gloor et al., 1993). Two fly equivalents of genomic DNA were subjected to restriction digest with NdeII (for inverse PCR), BamHI (for recovery of 5′ end), or EcoRI (for recovery of 3′ end of p[lacW]) vector. The digests were then ligated overnight at 4°C in a large volume (500 μl). For plasmid rescue, the ligated fragments (20 μl) were transformed into DH5α, and the resulting clones were analyzed by restriction digest and DNA sequencing. For inverse PCR, 10 μl of the ligation reaction (1:50 or 1:25 of an individual fly) was subjected to PCR with p[lacW] specific primers (Huang and Rubin, 2000). The products were analyzed by gel electrophoresis.
Staged larvae for growth, feeding, and labeling experiments

Pdp1<sup>p205</sup> balanced flies were allowed to lay on apple juice or grape juice plates at 22°C for 2 to 4 h. The plates were incubated at 22°C or 25°C until late embryos or larvae of the correct age were apparent. For selection of embryos and early larvae (first instar), Pdp<sup>p205</sup> homozygous and Pdp<sup>p205/TM3,St;</sup>Kr-Gal4,UAS-GFP heterozygous larvae were selected by the presence (heterozygous) or absence (homozygous) of GFP expression on a dissecting microscope fitted with a fluorescent source. Later, staged larvae were segregated based on size and verified by either expression of GFP or the tubby mutation, depending on the balancer used.

For gut acidification and alkalization assays, first and second instar larvae were fed yeast paste impregnated with approximately 0.2 to 0.4% bromphenol blue or phenol red pH indicators for 2 h (Dubreuil et al., 1998; Dubreuil et al., 2001). Larvae were dissected and photographed immediately.

For determining BrdU incorporation into replicating cells, larvae that were approximately 42 h AEL (±2 h) were floated on the surface of the 100 µg/ml bromodeoxyuridine (BrdU)/20% sucrose solution for 6 h (larvae were able to survive for longer than 24 h on this solution). The larvae (now at 48 h AEL) were then dissected in Ringer’s solution (physiological saline) so that brains and guts were intact. These were fixed and then subject to immunohistochemistry with the α-BrdU antibody (secondary α-mouse Rhodamine) and counterstained with DAPI to mark all nuclei (Galloni and Edgar, 1999).

Immunohistochemistry

Immunohistochemistry/Immunofluorescence of embryos was done according to standard protocols (Rothwell and Sullivan, 2000). Briefly, embryos were collected on fruit juice/agar plates, rinsed, fixed in 4–30% formaldehyde under heptane and methanol, and devitellinized. Embryos were stored under methanol until processing. Embryos were blocked and antibodies applied in PBS containing 3%BSA. The PDP1 rabbit antibody was used as unpurified serum at a concentration of 1/1000, and the mouse anti-MHC (courtesy of Dr. D. Kiehart) was used at a concentration of 1/50 to 1/100. The α-aknyrin antibody was courtesy of Dr. Ron Dubreuil (Dubreuil et al., 1998).

Embryos and larvae subjected to immunohistochemistry were photographed on a Zeiss microscope fitted with a fluorescent light source and filters capable of exciting fluorescein, rhodamine, and DAPI. Confocal images were taken on either a Zeiss LSM510 or PASCAL laser-scanning microscope at the UIC core facility. α-PDP antisera was used at a 1/1000 dilution, and horseradish peroxidase conjugated α-rabbit Ig (Sigma) was used at a 1/2000 dilution. Larval extracts were made by crushing 10 larvae in 2x SDS loading buffer including protease inhibitors but without reducing agent or dye. Once the larvae were macerated, β-ME and dye were added, and the entire extract boiled for 5 min. Western blots were performed according to standard protocols.

Mosaic analysis

Mosaic analysis was performed using the FLP/FRT method (Xu and Rubin, 1993). The relevant transgenic stocks were obtained from the Bloomington stock center. The Pdp1<sup>p205</sup> allele was recombined onto the P<sup>1</sup><sup>cyO;FRT80B</sup> (designated FRT 80B) chromosome using standard genetic crosses. Pdp1<sup>p205</sup> FRT 80B recombinant chromosomes were selected for resistance to 0.3 mg/ml G418, loss of the w<sup>−</sup> marker (from the piM transgene on the starting FRT chromosome), and the presence of the Pdp1 mutation. To generate clones, y,w,hsFLP<sup>22</sup>; Ubi-GFP<sup>p1</sup>,Pdp<sup>p205</sup> females were mated with w; Fdp<sup>p205</sup>FRT 80B/TM6B,Tb males. Progeny were heat shocked for 1 h at 37°C, either as embryos from an overnight collection for induction of clones in larval salivary glands and fat body or as first and second instar larvae for clones in imaginal tissues. Tissue from Tb<sup>−</sup> third instar larvae was dissected and examined.

Clonal areas in imaginal tissues were measured as has been previously described (Neufeld et al., 1998; Frolov et al., 2005). Briefly, imaginal discs were imaged using a Zeiss inverted microscope with apotome; twin spot clones were then outlined, and the clonal areas in pixels were measured using the histogram function of Adobe Photoshop.

BrdU incorporation experiments: to be able to simultaneously detect BrdU incorporation and GFP fluorescence in mitotic clone samples, the method was modified as per Beall et al. (2002) such that DNase I treatment rather than acidification by HCl was used to make the BrdU accessible to the anti-BrdU antibody.

Results

Pdp1 mutations

The Pdp1 locus at chromosomal location 3L-66A is flanked by genes encoding Henna (Hn), clock (clk), pebble (pbl), and sunray driver (syd). A null mutation at the Pdp1 locus was generated by P-element-mediated mobilization of the l(3)SO71411 line. This P-element was mapped to approximately 4 kb downstream of the 3′UTR of the Pdp1 gene, between Pdp1 and clock. Several mutations were generated by this scheme and one, Pdp1<sup>p205</sup>, was identified as a mutant allele of Pdp1 by inverse PCR, DNA sequencing, and complementation analyses with large deletions that remove the Pdp1 gene (Fig. 1A). The other deletions that were generated removed a larger region surrounding the Pdp1 locus, including genes known to be required for embryonic viability such as pbl. The deficiency generated in the Pdp1<sup>p205</sup> flies encompassed approximately a 55-kb region that spans from the original location of the SO71411 P-element, which remained intact at its original location at the 5′ end of the element (3′ end of the Pdp1 gene), to just upstream of the first exon of the Pdp1 gene. The pbl, syd, and clk genes were unaffected by this deletion as determined molecularly by inverse PCR and Southern blotting and functionally confirmed by complementation analysis. The deletion removed genomic DNA to within 70 bp of the putative open reading frame CG8294. No embryonic expression has yet been identified from this putative ORF. Thus, the Pdp1<sup>p205</sup> deletion removes only the Pdp1 gene. Furthermore, the expression of PDP1 in Pdp1<sup>p205</sup> embryos was examined by confocal microscopy using a polyclonal antibody against PDP1 (Cyran et al., 2003). As seen in Figs. 1B and C, PDP1 protein is not detectable in Pdp1<sup>p205</sup> embryos.

The findings reported here focus on our analysis of the Pdp1<sup>p205</sup> mutation, but we have also obtained and examined the phenotypes of two independently isolated mutant alleles of Pdp1. The Pdp1<sup>198</sup> mutation, which was generated in our EMS mutagenesis screen, gave a less severe phenotype than the Pdp1<sup>p205</sup> mutation. It also was growth retarded (see below), but a few larvae formed pupae and when dissected from their pupal case resulted in small and viable adults that died shortly thereafter. A second allele generated by Kalpana White, which we subsequently named Pdp1<sup>7A</sup>, had the same phenotype as Pdp1<sup>p205</sup>.

Pdp1<sup>p205</sup> larvae are growth delayed/arrested

Although PDP1 is expressed extensively throughout embryogenesis (Lin et al., 1997), the development through this stage appeared normal, and the embryos hatched into normal looking motile larvae. Soon after hatching, however, the
mutant larvae showed a dramatic lag in their growth compared to heterozygous siblings when reared on either grape juice plates supplemented with yeast or cornmeal/molasses media. Pdp1p205 larvae and their heterozygous siblings had similar hatching rates and similar motility and viability into the first larval instar. In addition, Pdp1p205 larvae and heterozygous larvae were the same size as newly hatched first instar larvae (24 h after egg laying, 24 h AEL) (Fig. 2). However, after hatching, the Pdp1p205 larvae were severely arrested in their rate of growth. At 5 days AEL, there was a dramatic difference in the size of the heterozygous larvae versus the Pdp1p205 larvae (Fig. 2). The Pdp1p205 larvae grew only slightly in overall size, whereas their heterozygous siblings nearly completed the 200-fold growth of a normal third instar larva. Despite their lack of growth, the Pdp1p205 larvae could survive for long periods of time as first, second, or even third instar larvae, with each instar lasting for extended periods of time (many days to over a week) compared with wild type. Larvae routinely survived for longer than 3 weeks, and some larvae lived for 30 days. We also noted that the Pdp1p205 larval phenotype was influenced by the nutritional condition of the media since a small number of Pdp1p205 larvae were able to pupate and began to eclose when raised on media supplemented with 25% sucrose and yeast as opposed to larvae raised on grape juice plates (or cornmeal/molasses) supplemented only with yeast which never pupated or reached adulthood. Sucrose levels affect the insulin-signaling pathway, and larvae deprived of amino acids usually die soon after deprivation but if supplied sucrose can survive for several days (Britton and Edgar, 1998). It was difficult to assign a lethal stage to Pdp1p205 larvae since there was attrition throughout the different stages of larval development, and the lifespan of some of the mutant larvae was as long as their heterozygous counterparts that progressed through metamorphosis and into adulthood. We thus refer to the phenotype as a growth delayed/arrested phenotype, although under normal growth conditions, these larvae do not undergo metamorphosis and are thus larval lethal.

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**Fig. 1.** The Pdp1 locus and Pdp1p205 deletion. (A) The Pdp1 locus is shown relative to the other genes in the region and several putative ORFs (designated CGxxx) that have been identified in this region by the Genescan program at the Berkeley Drosophila Genome Project. An enlargement of the area immediately surrounding the Pdp1 locus depicts the position of the (3) SOT1411 P-element used to generate the Pdp1p205 deletion. The deletion begins at the end of the P-element (the entire P-element is intact) and removes the entire Pdp1 gene (brackets) up to −1626 bp relative to the start site of transcription. The DNA sequence at the breakpoint is indicated. The deletion does not remove any other known genes and removes approximately 55 kb of genomic DNA. Panel B shows confocal sections (1 μm) of a wild-type stage 16 embryo that is stained for PDP1 (green) and Myosin Heavy Chain (MHC) (red). Because PDP1 is a transcription factor, most of the PDP1 staining is nuclear. Panel C shows a confocal section (1 μm) of a similarly staged Pdp1p205 embryo that is stained for PDP1 (absent) and MHC (red). MHC staining marks the visceral mesoderm that encases the gut and the somatic body wall muscles underlying the epidermis. There is also some non-specific MHC staining of the yolk in this more medial section of the embryo in panel C. All embryos are shown with a lateral view (anterior to the left and dorsal side up).
In addition to the growth delay, the ecdysis of the Pdp1p205 larvae is also delayed as indicated by the late appearance of mouth hook apparatus, however, the larvae do molt and progress through the larval instars. The Pdp1p205 larvae did not have second instar mouth hooks until 96 h AEL (on grape juice supplemented with yeast), whereas the heterozygous or wild-type larvae began to develop second instar apparati by 48 h AEL. This phenotype could not be rescued by the addition of exogenous ecdysone (data not shown), indicating that an ecdysone deficiency is not the primary cause of the growth delay/arrest.

The gut functions normally in Pdp1p205 larvae

In addition to the growth delay, the ecdysis of the Pdp1p205 larvae is also delayed as indicated by the late appearance of mouth hook apparatus, however, the larvae do molt and progress through the larval instars. The Pdp1p205 larvae did not have second instar mouth hooks until 96 h AEL (on grape juice supplemented with yeast), whereas the heterozygous or wild-type larvae began to develop second instar apparati by 48 h AEL. This phenotype could not be rescued by the addition of exogenous ecdysone (data not shown), indicating that an ecdysone deficiency is not the primary cause of the growth delay/arrest.

The growth delayed/arrested phenotype of Pdp1p205 larvae suggested that they might have a nutritional defect that could be caused by abnormal gut function. The guts of Pdp1p205 embryos and larvae looked morphologically wild type (Figs. 2 and 3), however, it was unclear from these assays whether the alimentary organs retained normal function. For example, pumpless mutant larvae, which are also growth arrested, wander away from their food inappropriately and fail to swallow a good portion of their food (Zinke et al., 1999). Therefore, it was necessary to determine if Pdp1p205 larvae have normal food attraction behavior and have a functioning digestive system. Pdp1p205 larvae responded to food normally; they congregated around yeast paste in the center of an apple juice agar plates, as do wild-type larvae; and feeding with yeast dyed with Fast green showed that these larvae ingested and excreted food (data not shown). Thus, Pdp1p205 larvae are attracted to and eat food normally.

To further assess the function of the gut, several assays were performed that tested the compartmentalization of the gut and the integrity of the acid secreting cuprophilic cells. Mutations in the labial and α-spectrin genes, which both cause a larval lethal phenotype, are able to disrupt normal gut function by abolishing the ability of the cuprophilic cells to secret acid into...
the lumen of the gut (Dubreuil et al., 2001). In the larval gut, the anterior part of the midgut is acidic, while the posterior part of the midgut is more alkaline. The presumptive cuprophilic cells express PDP1 in the embryo, as do other regions of the midgut, and a Pdp1 enhancer is expressed in the cuprophilic cells in the larva (Reddy et al., 2000). To determine if the Pdp1P205 larvae had normal acidification and alkalization of the gut, 24 h AEL wild-type and Pdp1P205 larvae were fed yeast paste with bromphenol blue or phenol red for several hours. These dyes served as color indicators of the relative acidity and alkalinity in different regions of the gut. For example, in α-spectrin mutants, who die as first instar larvae, the cuprophilic cells fail to acidify the anterior midgut, and ingested bromphenol blue dye never turns yellow in the middle midgut (Dubreuil et al., 1998). In all cases, the guts of Pdp1P205 larvae were normal for acidification and alkalization, indicating overall normal partitioning and functioning of the gut, as well as normal cuprophilic cell function (Fig. 3). The bromphenol blue dye turned yellow in the middle midgut of both wild-type and Pdp1P205 larvae, indicating functioning acid secreting cuprophilic cells in the mutant larvae (Fig. 3A). Also, the phenol red dye turned red in the posterior midgut, indicating that alkalization is normal in Pdp1P205 larvae. To visualize and morphologically assay the cuprophilic cells, the larvae were fed 1% copper sulfate in a corn meal-based food for 1 h, dissected, and photographed immediately. The cuprophilic cells in both the wild-type and mutant larvae took up copper (orange glow under UV light), and the morphology of these cells was normal (Fig. 3B). Taken together, these data indicate that the regional specification, acidification, and cuprophilic cells are normal in the Pdp1P205 mutant larvae.

While the alimentary canal of the Pdp1P205 larvae appeared to be normal by compartmentalization and feeding assays, it was important to further analyze different cell types in the gut. Ankyrin, a cell surface protein that is expressed in the endoderm, is a good marker for gut cells (Dubreuil et al., 1998). Using ankyrin antibody to analyze gut cells, the dissected Pdp1P205 larval guts looked normal when compared to their heterozygous counterparts, whether comparing size-matched or age-matched larvae (not shown). Also comparing α-ankyrin staining with DAPI staining of age-matched dissected guts further supported this finding. Although the cells in the gut are considerably smaller in Pdp1P205 larvae, their gross morphology is normal, and the number of cells appeared to be roughly the same in the wild-type and mutant larvae.

Two other tissues examined which express PDP1 in the embryo and larva were the fat body and the malpighian tubules. The overall morphology of both tissues appeared normal in the Pdp1P205 larvae. Immunofluorescence assays using an antibody to Serpent, which is expressed in the fat body, showed normal fat body staining as well as normal developmental progression (Hayes et al., 2001), but it is of course possible that they are not functioning normally at the molecular level. Also, the malpighian tubules of the Pdp1P205 larvae accumulated uric acid crystals, a waste product of protein digestion, indicating that they functioned normally.

Pdp1P205 larvae exhibit reduced mitoses and endocycles

Growth delay/arrest can be caused by starvation, cell cycle defects, problems with edysone signaling, or failure to coordinate nutritional environment with growth. Since the growth problems of Pdp1P205 larvae did not appear to be due to lack of 20-HE production/signaling and other experiments indicated that the Pdp1P205 larvae are able to digest and utilize food, it was necessary to establish that the larvae were not starving for other reasons, such as failure to take up certain amino acids. A characteristic of larvae deprived of amino acids is that larval cells stop endoreplicating within 12 h of amino acid withdrawal leading to a growth arrest phenotype (Edgar et al., 2001; Edgar and Orr-Weaver, 2001).

To determine if the Pdp1P205 larvae undergo normal replication, staged mutant and wild-type larvae that were 42 h AEL were fed the dTTP analog BrdU for 6 h. Typical results are shown in Fig. 4. Cells showing BrdU incorporation in the brain (arrows in Figs. 4B, D, F, and H) are mitotic cells (larval neuroblasts) and served as a positive control for these experiments, since these cells continue cycling, once started, even after amino acid withdrawal (Britton and Edgar, 1998). The cells showing BrdU incorporation in the gut (brackets in Figs. 4B and D and at higher magnification in panels I–P below) are endoreplicating cells. Cells in the gut of Pdp1P205 larvae, therefore, clearly undergo endoreplication, indicating that they are not starving. However, by comparing wild-type and Pdp1P205 larvae, the percentage of cells undergoing replication is reduced in Pdp1P205 larvae from approximately 27% in the wild type to 17% in the mutant. In this context, it should be noted that Pdp1P205 larvae are already smaller than their wild-type counterparts at this time point (48 h AEL), and thus at the same magnification of both, a larger area of the Pdp1P205 gut is seen, and the individual cells are smaller (Figs. 4B, D). It is important also to note that incorporation of BrdU into wild-type cells was optimal in less than 3 h of incubation in wild type, whereas in Pdp1P205 larvae, BrdU incorporation could not be visualized in 3 h and reached a comparable level of incorporation only after 6 h of incubation. This difference in time to reach a comparable level of incorporation indicates that although DNA replication is occurring in endoreplicating cells of Pdp1P205 larvae, it is occurring at a much slower rate compared to wild type. The number and size of the neuroblasts made it difficult to accurately count and quantitate the level of BrdU reduction in the brain. Nevertheless, it is obvious that in contrast to the relatively modest effect on the incorporation of BrdU in endoreplicating cells, there was a massive reduction in the number of larval neuroblasts incorporating BrdU in the brain (arrows in panels F and H) or in the imaginal cells of the foregut (arrows in J and L) in Pdp1P205 larval. All of these cell types undergo a mitotic rather than an endoreplicative cycle.

Next, larvae that were 72 h AEL were examined using the same assay. At this stage of larval development, the wild-type larvae are considerably larger than the Pdp1P205 larvae, and considerably more cells are incorporating BrdU
The mutant $Pdp1p205$ larvae still incorporated BrdU in the endoreplicating cells of the gut (brackets in Figs. 5A, I, M, and Q) and in the mitotic cells of the brain (arrow in Fig. 5E), but the levels of incorporation were dramatically reduced in both the endoreplicating cells (from approximately 61% in wild type to 19% in the mutant) and mitotic cells in comparison to wild type (Fig. 5A arrowheads, Figs. 5C and E, and 6D). For instance, there are only a few sporadic cells endoreplicating in the anterior and middle portion of the midgut (Figs. 5I and M) and a significantly reduced number in the posterior midgut (Fig. 5Q).

By examining the tissues that proliferate the most rapidly at 72–78 h AEL, the imaginal discs and the neuroblasts of the brain, it is most evident that the $Pdp1p205$ larvae are severely compromised in their rate of mitoses. Although the imaginal discs are present (see eye-antennal disc in Fig. 6C), and there is some mitotic activity in the brain (Fig. 6D), the overall sizes of these organs were much smaller than their heterozygous counterparts, and BrdU positive cells were absent or greatly reduced in number, suggesting an absence or greatly reduced rate of proliferation. Similarly, striking differences were observed in endoreplicating tissues such as fat body and salivary glands. The nuclei of the latter, which...
Fig. 5. BrdU incorporation in 72 h AEL heterozygous wild-type and Pdp1p205 larvae. Panel A shows the size difference between wild-type and Pdp1p205 larvae at 72 h AEL. On the left (B, C, F, G, J, K, N, O) are images of a 72 h AEL wild-type larva gut stained for DAPI or BrdU as indicated. On the right (D, E, H, I, L, M, P, Q) is a dissected gut from a Pdp1p205 larva stained for DAPI or BrdU as indicated. The brackets mark comparable regions of endoreplicating cells in the midgut incorporating BrdU labeling are shown at higher magnification in panels F–Q. The arrows point to the brain. Since the larvae are not the same size at this developmental time point, there is a noticeable difference in the number of cells shown for wild type versus the mutant, with the panels of the mutant larva showing a greater proportion of the tissue examined. Panels B–E show staining and incorporation in larval brains (arrows in panels A, D, and E). Note that because of the size difference, two lobes are visible in the mutant versus one in the wild type. Panels F–I correspond to the top brackets in panel A and show the anterior midgut just posterior to the gastric caeca. Panels J–M correspond to the second (from the top) bracked regions of the midgut and show the staining of the middle midgut (note the brain of another larvae showing in the heterozygote panels). Panels N–Q correspond to the third bracked regions in panel A showing staining in the posterior midgut just anterior to the mapighean tubules.
normally endoreplicate to 1024C, were much smaller in 
Pdp1p205 than in wild type (compare Figs. 6C and E). Furthermore, virtually no BrdU incorporation was detected in the fat body and salivary glands of the 
Pdp1p205 larva (Fig. 6D), while in the wild-type larva, more than 50% of the salivary gland cells were positive (Fig. 6F). In mutant larvae that are more than 5 days old, the incorporation of BrdU was also very slow or non-existent (data not shown), but it was difficult to evaluate this data since there is no wild-type comparison.

In the midgut of wild-type third instar larvae, there are two types of cells that are easily distinguished by DAPI stain; the large polytene (endoreplicating) cells and the groups of mitotic cells that make up the so-called mitotic islands or imaginal nests which proliferate late in third instar (Fig. 6G, arrows). It is the mitotic islands that will give rise to the adult gut epithelium. In 
Pdp1p205 larvae that are 4.5 or 13 days AEL, these cells are virtually absent (Fig. 6H). Instead, there is typically a single cell, perhaps a precursor that has failed to divide, in place of each nest (arrows in Fig. 6H). Overall, these
data confirm that the mutant larvae are not growth arrested because of total starvation, which would lead to an immediate cessation of endoreplication. However, the severely reduced mitosis in brain and imaginal cells as well as the slowed endoreplication indicates that the cause of the growth delay/ arrest is due to defects in the control of the mitotic and endoreplicative cycles.

**PDP1 is sensitive to nutritional environment**

The growth defects in the *Pdp1P205* larvae prompted us to determine if supplementing the growth medium with either protein sources (2% yeast extract or peptone) or sugars (25% sucrose) would ameliorate the *Pdp1P205* larval phenotype. Wild-type and *Pdp1P205* larvae were allowed to grow on either supplemented or unsupplemented 30% apple juice/agar plates with yeast paste (all growth conditions had yeast paste) at 25°C. Wild-type larvae survived, grew, and pupated equally well on supplemented or unsupplemented plates (puparium formation at 5dAEL). *Pdp1P205* mutants were unaffected by protein supplementation but grew much better on the 25% sucrose supplemented plates. On unsupplemented plates, *Pdp1P205* larvae remained as larvae for up to 30 days, but on the sucrose supplemented medium, many of the mutant larvae (~20%) were able to form pupae at 14 to 16 days AEL (still delayed compared to wild type). A few were able to reach adulthood, although these adults needed to be dissected from their pupal cases and died soon thereafter. These results indicate that extra dietary sugar can partially rescue the *Pdp1P205* mutation, and that the mutant larvae are especially sensitive to their nutritional (sugar) environment compared to wild-type larvae.

Since *Pdp1P205* larvae were more sensitive to sucrose levels than their wild-type siblings, we postulated that PDP1 might play a role in sensing environmental conditions and thus interact with signaling pathways that couple environmental conditions with cell cycle progression and endoreplication (Edgar et al., 2001; Britton et al., 2002; Zhang et al., 2000). If so, PDP1 expression might be affected in response to nutritional conditions in order to cope with such changes. To test this hypothesis, PDP1 protein was measured from wild-type larvae that were either fed or starved for 48 h. Rabbit α-PDP1, which recognizes all isoforms of PDP1, was used to detect relative levels of PDP1 protein. The protein isotypes, PDP1-φ and PDP1-ε, are sensitive to the nutritional environment.

**PDP1 acts in a cell non-autonomous fashion to influence the cell cycle**

Our studies showed that the growth and/or proliferation of virtually every cell type is adversely affected in *Pdp1* mutant larvae even though PDP1 expression is not detectable in many of these tissues. For example, immunocytochemical analysis in larval brains shows that PDP1 is expressed in a small number of neurosecretory cells, but not in the entire neuroblast population (Cyran et al., 2003). However, in *Pdp1P205* larvae, a large number of larval neuroblasts exhibit a delayed/or deficient mitotic phenotype. Similarly, although PDP1 is not expressed in salivary glands, endoreplication is reduced or absent in these cells in mutant larvae by 72 h AEL. These findings suggest that PDP1 is not acting in a cell autonomous fashion. To examine this directly, we used the FLP-FRT system of recombination (Xu and Rubin, 1993) to generate somatic clones of *Pdp1P205* mutant cells.

*Pdp1P205* mutant clones were readily observed in all third instar larval imaginal tissues, indicating that the mutant cells survive and proliferate. To evaluate their rate of growth relative to the surrounding cells, we examined *Pdp1P205* mutant clones...
and their corresponding wild-type twin spots in third instar larval wing discs, which have been shown to be ideally suited for this type of analysis because the cells are proliferating asynchronously, the clonal populations of cells tend to stay together, and the surface of the disc is relatively flat (Neufeld et al., 1998; Frolov et al., 2005). We found that the twin-spot Pdp1<sup>p205</sup> mutant and wild-type clones grew to the same sizes, and that the density of nuclei was similar in homozygous Pdp1<sup>p205</sup> mutant, wild-type, and heterozygous cells (Figs. 8A, B). Furthermore, when the discs were labeled with BrdU to visualize cells in S-phase, no differences in either the proportion of cells in S-phase or the efficiency of BrdU labeling were observed between Pdp1<sup>p205</sup> mutant and wild-type cells (Fig. 8C). These findings show that within the context of a heterozygous animal, diploid cells in Pdp1<sup>p205</sup> mutants proliferate and grow normally. To determine whether the striking retardation of growth in the endoreduplicating cells of Pdp1<sup>p205</sup> mutants is also non-autonomous, mitotic clones were induced in these tissues during embryogenesis. We examined salivary glands and fat body and found that both the size and appearance of the homozygous Pdp1<sup>p205</sup> mutant nuclei and cells were indistinguishable from that of the neighboring heterozygous and homozygous wild-type cells (Figs. 8D, E and not shown). Taken together, the results from analysis of Pdp1<sup>p205</sup> mutant cells in mosaic animals demonstrate that PDP1 acts non-autonomously to regulate cell proliferation and tissue growth.

**Discussion**

Pdp1 is dispensable for embryogenesis despite the fact that Pdp1 is expressed in several tissues during embryogenesis. Tissues such as the gut, fat body, and muscle, which express PDP1 at high levels as they differentiate, have normal morphology in Pdp1<sup>p205</sup> embryos, and all appeared to function normally in Pdp1<sup>p205</sup> larvae. Although maternally supplied stores of mRNA and proteins can obfuscate loss-of-function embryonic phenotypes by compensating for loss of zygotic function, this does not seem to be the case here as maternally supplied Pdp1 messenger RNA or protein is not detected in early embryos (Reddy et al., 2000). Therefore, either Pdp1 is not necessary for embryonic development, the effects of Pdp1 loss-of-function are subtle and not detected in our assays, or Pdp1 function is being compensated for by another gene. This latter possibility is consistent with previously reported deletion analysis of PDP1 binding sites.

![Fig. 8. PDP1 acts non-autonomously on cell growth and proliferation. Clones of Pdp1<sup>p205</sup> mutant cells were induced by mitotic recombination. Panel A shows a third instar larval wing disc with examples of homozygous mutant Pdp1<sup>p205</sup> clones identified by the absence of GFP, and their homozygous wild-type twin spots, visualized by an increased GFP signal within the context of a heterozygous background (clones are outlined in white; DAPI-staining to visualize nuclei, red; GFP fluorescence, green). The density of nuclei in the three populations of cells was the same indicating that the mutant cells were of normal size. The histogram in panel B displays the sizes of Pdp1<sup>p205</sup> mutant clones and their corresponding wild-type twin spots for fifteen pairs of clones from independent wing discs. The bars are ordered according to the size of the wild-type twin spots. No differences were detected between the sizes of Pdp1<sup>p205</sup> mutant and wild-type cells or twin spot clones. To evaluate cell cycle progression in mitotic cells, dissected imaginal discs in which Pdp1<sup>p205</sup> mutant clones had been induced were labeled with BrdU to visualize cells in S-phase. Panel C shows a representative example of a mutant wing disc clone outlined in white (BrdU incorporation, red; DAPI-staining, blue; GFP fluorescence, green). No differences in either the proportion of cells in S-phase or the efficiency of BrdU labeling were observed between Pdp1<sup>p205</sup> mutant and wild-type cells. To assess the effects of lack of Pdp1 function on endoreduplicating cells, mitotic clones were induced during embryogenesis. Panels D and E show two pairs of homozygous Pdp1<sup>p205</sup> mutant (arrows) and wild-type (dashed circles) cells in a third instar larval salivary gland which were only distinguishable by the differences in GFP fluorescence (DAPI-staining, red in panel D, white in panel E; GFP fluorescence, green in panel D). Scale bars: in panel A, 0.02 mm for panels A, C; in panel D, 0.05 mm for panels D, E.
in the context of the larger muscle enhancer of the Tml gene (Lin et al., 1997).

The Pdp1P205 mutant embryos develop and hatch normally and become larvae which are able to crawl and eat, indicating normal muscle function and at least rudimentary gut function. However, these Pdp1P205 larvae are severely growth delayed compared to their heterozygous counterparts. This Pdp1P205 larval phenotype is very similar to the phenotype of amino acid starved larvae and larvae derived from dTOR and slimfast mutants, all of which affect larval growth through altered endoreplication. The growth of the larva is dependent upon its nutritional state. Wild-type larvae starved of nutrients (amino acids) have retarded growth that mimics the Pdp1 phenotype, although Pdp1P205 larvae do crawl and eat. The coordination of growth with nutritional conditions is most readily observed in the endoreplicating cells of the larva. In larvae, the initial uptake of amino acids from an exogenous food source stimulates both endoreplicative and mitotic cell cycles (Britton uptake of amino acids from an exogenous food source). Growth with nutritional conditions is most readily observed in these cells continue cycling after food withdrawal. Thus, a initial pulse of amino acids to stimulate mitotic cycling since amino acids is necessary to maintain endoreplication, as evidenced by complete loss of endoreplication 24 h after food withdrawal. Mitotic cells, on the other hand, require only an initial pulse of amino acids to stimulate mitotic cycling since these cells continue cycling after food withdrawal. Thus, a fundamental difference between Pdp1P205 larvae and amino acid starved larvae is that in Pdp1P205 larvae, endoreplication does occur after hatching as assayed via BrdU incorporation. In wild-type and Pdp1P205 larvae that are at 48 h AEL (late first instar for wild type), endoreplication is occurring in three locations within the gut during the 6-h BrdU incorporation time.

Since endoreplication does occur, these larvae are not starving per se; they take up amino acids. Furthermore, they appeared to have normal gut functions, and adding amino acids to the diet of Pdp1P205 larvae did not improve growth or viability. However, although endoreplication is occurring in first instar Pdp1P205 larvae, it is progressing at a much slower rate since BrdU incorporation could not be readily detected after a 3-h period of incubation even though these were optimal conditions for wild-type larvae. Instead, a two- to three-fold longer incubation is required in Pdp1P205 larvae to reach a comparable level of BrdU incorporation as in wild-type larvae. Thus, we conclude that although larval cells are endoreplicating in young Pdp1P205 larvae (48 h AEL), they are already doing so at a much slower rate, leading to a severely reduced number of endocycles. This is unlike older larvae where little or no endoreplication is observed. Since adding exogenous sucrose improved upon the phenotype, we suspect that although Pdp1P205 larvae may be getting sufficient nutrients in their diet, they may suffer from a metabolic defect that results in altered carbohydrate metabolism. Such larvae may not breakdown glucose or transport glucose efficiently and thus may grow slowly and experience developmental delays which may be partly overcome by the increasing the sugar (sucrose). It is known, for instance, that insulin signaling from the brain promotes mitosis and endoreplication and is particularly sensitive to carbohydrate levels (Garofalo, 2002).

Therefore, it is possible that extra stimulation of the insulin-signaling pathway is able to partially bypass the Pdp1 null phenotype.

In wild-type animals, DNA synthesis (as visualized by BrdU incorporation) is also occurring in the mitotic imaginal cells of the brain, gut, and discs that will give rise to adult organs. In young Pdp1P205 mutant larvae (48 h AEL), BrdU incorporation into these cells is dramatically reduced and/or apparently absent in some cases. This lack of cell cycle progression carried over into older larvae (72 h AEL), a timeframe during which endoreplication had essentially ceased in the mutants even though midgut, salivary gland, and fat body cells are actively undergoing endoreplication in wild-type larvae. Also striking are the severely reduced levels of mitotic cell types including the imaginal cells of the midgut, brain neuroblasts, and imaginal disc cells. Thus, in comparison with endoreplicating cells, mitotically active cells in the larva seem to be especially sensitive to the loss of PDP1 function.

Pdp1P205 mutants also have abnormal circadian rhythm (Cyran et al., 2003). It was shown previously that PDP1-ε is a positive regulator of Clock and is part of a second feedback loop in the circadian clock mechanism of cyclic output regulation (Cyran et al., 2003). Thus, Pdp1P205 mutants do not express TIM, PER, or PDF in the pacemaker cells of the brain (Cyran et al., 2003; Gekakis et al., 1995; Baylies et al., 1992; Sehgal et al., 1995). In mice, it has been shown that the circadian clock controls expression of cell cycle genes that regulate mitosis (Matsuo et al., 2003). Moreover, in vertebrates and Drosophila, hormonal levels regulated by circadian output control nutritional and behavioral responses (Cassone, 1998; Ahima et al., 1998). In Drosophila, the clock-regulated output gene takeout (to) has been shown to be directly linked to feeding and starvation and has been proposed to contribute to the metabolic and behavioral changes in response to the absence of food (Sarov-Blat et al., 2000). This raises the intriguing possibility that Pdp1 might integrate circadian rhythm with the nutritional state of the organism. For instance, PDP1 might regulate clock output genes that determine cyclic feeding behavior or metabolism. PDP1 might also help regulate cyclic mitogenic and/or nutrient sensing pathways that coordinate growth with nutritional conditions. If true, then PDP1 itself might be regulated by nutritional conditions.

Given that Pdp1P205 larvae are sensitive to nutritional environment, specifically sugar levels in the growth medium, we analyzed the isotype profile under starvation conditions in wild-type larvae. Indeed, upon starvation, the PDP1-ε isotype is upregulated. This is similar to the to gene which is also upregulated during starvation (Sarov-Blat et al., 2000). Given the growth phenotype of Pdp1P205 larvae and the PDP1 isotype switching that occurs under starvation conditions, it is conceivable that this sensitivity to nutritional conditions is fundamental and necessary to the function of the PDP1 protein. This is especially interesting since the PDP1-ε isofrom is not expressed in embryos and may indicate that this isoform is necessary in larval/adult life for the purpose of sensing nutritional environment. In the embryo, such a sensor is unnecessary because the embryo has all nutritional requirements supplied by its yolk and therefore has no nutritional
interaction with the environment. The nutritional regulation of PDP1 is also provocative given that the homologous vertebrate PAR proteins are involved in the circadian regulation of genes that are involved in metabolism (Babajko and Groyer, 1993; Lavery and Schibler, 1993; Lavery et al., 1999; Wuarin et al., 1992; Roesler et al., 1992). This link between Pdp1, vertebrate PAR proteins, and circadian rhythm is further reinforced by the observation that, in addition to the upregulation of the PDP1-ε isoform in starved larvae, we typically observe an upregulation of the PDP1-ε isoform as well. Since PDP1-ε is expressed in the clock cells where it regulates clk and other clock output genes, it is possible that nutritional entrainment of the molecular clock in Drosophila could occur through this isotype.

Taken together, the data suggest that PDP1 in the larva may be acting as a transcriptional regulator of growth and proliferation through sensing the nutritional environment. Indeed, it has been shown recently that VRI, which together with PDP1 regulates the Clock gene, is required for normal cell growth and proliferation (Szuplewski et al., 2003). VRI, however, appears to act cell autonomously, whereas PDP1 does not. The pleiotropic phenotype exhibited by Pdp1p205 larvae suggested that the growth defects are not cell autonomous since there is no detectable PDP1 expression in some of the affected tissues. Mosaic analysis confirmed that the effects of the Pdp1p205 mutation on cell growth and proliferation are non-autonomous. A possible model to explain how PDP1 could function to integrate the nutritional state with the regulation of growth and proliferation is shown in Fig. 9. Since the effects of PDP1 are non-autonomous, the most likely possibility is that it exerts its effects through a diffusible factor(s). There are two organs in which PDP1 is expressed that are known to emit diffusible factors that affect mitosis, endoreplication, or both. First, the fat body is known to secrete mitogenic factors required for proliferation of cells in the imaginal disc. It has long been known that growing imaginal discs in culture require medium conditioned with fat body or fat body extracts (Britton and Edgar, 1998). The Imaginal Disc Growth Factors (IDGF) is a group of recently discovered peptides that are required for proliferation in the imaginal tissues and are expressed in the fat body (Kawamura et al., 1999; Bryant, 2001). There is no evidence that these factors impact endoreplication directly in the larva, but they do cooperate with insulin-like growth factors to promote endoreplication. The fat body can, however, regulate growth and endoreplication. Reducing amino acid uptake by the fat body or inhibiting dTGF signaling in the fat body inhibits growth and endoreplication in peripheral tissues perhaps through the regulation of transport protein genes in the fat body such as slimfast, which when inhibited leads to larval growth defects and reduced endoreplication (Columbani et al., 2003). Perhaps PDP1 is involved in regulating fat body functions associated with either IDGF, other mitogen expression, transport, or fat body response to amino acid levels.

PDP1 might exert its effect on insulin growth factor expression or its pathway(s) through signaling from the fat body. Insulin-like growth factors are expressed in neurosecretory cells in the brain, and the insulin-signaling pathway is implicated in regulating both proliferation and endoreplication (Britton et al., 2002). Ectopically expressing either the insulin receptor (InR) or Drosophila S6 kinase (dsS6K), two proteins in this pathway, is able to relieve endoreplication defects seen in starved larvae (Zhang et al., 2000). Mutations in several factors in this pathway, such as dsS6K, InR, InsP3 and dTOR, have larval growth defects (Britton et al., 2002; Zhang et al., 2000; Neufeld, 2004; Oldham et al., 2002; Garofalo, 2002; Schmelzle and Hall, 2000; Jacinto and Hall, 2003). Insulin signaling is also implicated in the proliferation of the imaginal disc cells. The most downstream factors in this pathway regulate the S-phase promoting factor E2F and ectopically expressing E2F is also able to rescue endoreplication defects seen in starved larvae or larval growth defective mutants (Asano et al., 1996; Britton and Edgar, 1998; Caldwell and Datta, 1998).

Since the effects of the Pdp1 mutation are pleiotropic, even though PDP1 is not expressed in all affected tissues, our working model places PDP1 downstream of nutritional signals and upstream of mitogenic signals. In this model, a nutritional signal could be received by either the fat body or the brain and, either directly or indirectly, affect PDP1 regulation of the production of further signaling molecules. PDP1 could either be required for proper organ function or could regulate factors involved in nutritional homeostasis and signaling. In the case of the fat body, PDP1 could modulate the production of IDGFs or other circulating mitogenic factors, while in the brain, PDP1 could conceivably modulate IGF production. Furthermore, incorporating data from PDP1 regulation of the circadian clock, it is possible that PDP1 can serve as a link between nutritional state, growth, and circadian rhythm.
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