calderón encodes an organic cation transporter of the major facilitator superfamily required for cell growth and proliferation of Drosophila tissues

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The adaptation of growth in response to dietary changes is essential for the normal development of all organisms. The insulin receptor (InR) signalling pathway controls growth and metabolism in response to nutrient availability. The elements of this pathway have been described, although little is known about the downstream elements regulated by this cascade. We identified calderón, a gene that encodes a protein with highest homology with organic cation transporters of the major facilitator superfamily, as a new transcriptional target of the InR pathway. These transporters are believed to function mainly in the uptake of sugars, as well as other organic metabolites. Genetic experiments demonstrate that calderón is required cell autonomously and downstream of the InR pathway for normal growth and proliferation of larval tissues. Our results indicate that growth of imaginal cells may be modulated by two distinct, but coordinated, nutrient-sensing mechanisms: one cell-autonomous and the other humoral.

KEY WORDS: Insulin pathway, Growth, Wing, Drosophila

INTRODUCTION

One of the most obvious differences between animals, even in the same systematic group, e.g. mammals, is size. However, little is known about the mechanisms that underlie the control of cell, organ or body size. The dimensions of an organ and an organism depend on the number of cells and on the size of each individual cell. In multicellular organisms, growth regulation depends on the integration of various genetic and environmental cues (Conlon and Raff, 1999; Stern and Emlen, 1999). Nutrient availability is one of the major environmental signals that affects growth and, as such, complex humoral responses ensure that growth and development are properly coordinated with nutritional conditions.

Multicellular organisms respond to nutrient availability through cell-autonomous and -non-autonomous mechanisms. Growth regulation through the latter occurs via the release of insulin-related growth factors from peripheral tissues. In Drosophila, ablation of several neurosecretory cells expressing insulin-like peptides causes a growth defect (Rulifson et al., 2002), and mice lacking an anterior pituitary expressing growth hormone are dwarves (Butler and Le Roith, 2001). Studies in Drosophila have highlighted a crucial role for the insulin receptor (InR) pathway in regulating the cell-autonomous response to nutrient availability (reviewed by Goberdhan and Wilson, 2003; Stocker and Hafen, 2000). The InR, a tyrosine kinase transmembrane receptor, induces phosphorylation of insulin receptor substrates (IRS), which activate a cascade of downstream effectors. In vertebrates, genetic manipulation of several elements of this pathway modulates tissue growth in vivo thus demonstrating that the insulin-like growth factor (IGF) pathway is required for growth (Efratstadias, 1998; Shioi et al., 2000; Shioi et al., 2002).

The InR downstream effectors PI3 kinase/Dp110 and target of rapamycin (TOR) exert some of their growth effects at the transcriptional level (reviewed by Neufeld, 2003). In yeast, TOR controls the expression of a broad group of genes that are involved in protein, lipid and nucleic acid metabolism (Beck and Hall, 1999; Cardenas et al., 1999). However, in multicellular organisms little is known about effectors of the InR pathway that are regulated transcriptionally. Here we identify calderón (cald; orct2FlyBase), which encodes an organic cation transporter of the major facilitator superfamily, as a downstream effector of the InR pathway in Drosophila. Loss of cald activity mimics the phenotype of mutations in the InR pathway during embryonic and adult development. Expression of calderón is positively regulated by the InR downstream effectors PI3 kinase/Dp110 and TOR, and its activity is required for TOR-mediated growth induction. Thus, calderón is a target of the PI3 kinase/TOR branch of the InR pathway required cell autonomously for insulin-mediated cell growth.

MATERIALS AND METHODS

Drosophila strains: Ultrathorax-Gal4 (Ubx-Gal4 in the text; M. Calleja and G. Morata, unpublished), engrailed-Gal4 (en-Gal4 in the text) (Tabata et al., 1995), patched-Gal4 (ptc-Gal4 in the text) (Wildler and Perrimon, 1995), MS-1096-Gal4 (Milan et al., 1998), Kruppel-Gal4 (Kr-Gal4 in the text) (Castelli-Gair et al., 1994), EP1072 (Bloomington Stock Centre), UAS-p35 (Hay et al., 1995), UAS-Rheb (a gift from Bruce Edgar) (Saucedo et al., 2003), UAS-dS6K (a gift from T. P. Neufeld), UAS-P13K92E-Dp1101095/1096 (a gift from P. Léopold) (Leevers et al., 1996), and brkMS-lacZ (Jazwinska et al., 1999) were used. H(PDelta2-3)HoP2.1 transposase and UAS-GFP are described in Flybase. The UAS-cald transgenic lines were generated by cloning the whole open reading frame [ORF (isolated by PCR of genomic fly DNA)] into the pUAST vector, using the BglII/XhoI cloning sites. The constructs were injected into y w1118 embryos, and stable lines were selected by rescue of the white phenotype. To distinguish hemizygous or homozygous mutant embryos from their heterozygous siblings, we used the balancer TM6b, AbdA-lacZ.

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Molecular localization of calderón-Gal4 and P-element mutagenesis

Using plasmid rescue, we cloned and sequenced the flanking genomic sequence 3’ of the PGawB element (Brand and Perrimon, 1993). The insert is located 422 bp upstream of the CG13610 transcription start. For the generation of P-element excisions, males homozygous for the P-gal4 insertion (Calgal-Gal4) were crossed with females carrying the H/PE1-2-3/HoP-2.1 transposase on the CyO balancer chromosome. Excisions of the P-gal4 transposon were selected by the loss of the white eye in the F1 progeny. Individual revertants were crossed with TM3/TM6B flies and balanced. PCR analysis was performed with individual stocks corresponding to a new complementation group. We used one primer located 480 bp downstream of the P-element insertion site and another primer located 330 bp upstream of this site: downstream primer: 5’-GCTGCTGCTCTGCG-AGGCCAGC-3’; upstream primer: 5’-GCAACTGACTTCTTGCGA-GTGCGCCGG-3’.

The mutants recovered corresponded to new insertions of 2.7 Kb (R106), 9 Kb (R107) and 50 bp (R161) in the same place, 11 bp downstream of the original LP1 insertion site.

Analysis of the calderón R161 developmental delay

Embryos that were 0-24 h old were collected, and the development of heterozygous calderónR161/+ and homozygous calderónR161 animals was analyzed. 77 heterozygous calderónR106/+ larvae required 5-6 days to reach the pupal stage and 5 days to eclose as adults. Ten homozygous calderónR161 larvae required 8-9 days to reach the pupal stage and 6 days to eclose as adults. Homozygous calderónR165 individuals were identified by the absence of the Hemeral dominant marker of the balancer TM6b.

Genetic mosaics

The following Drosophila strains were used to generate loss-of-function clones: FRT82B calderónR107/TM6B; FRT82B calderónR106/TM6B; FRT82B calderónR161/TM6B; FRT82B calderónR107/TM6B; hs-FLP122; FRT82B arm-lacZ; hs-FLP122; FRT82B ubiquiGFP; y+w hs-FLP122; FRT82B arm-lacZ; y+w hs-FLP122 tub-Gal4:UAS-GFP; FRT82B tub-Gal80.

The FLP/FRT technique (Xu and Rubin, 1993) was used to generate loss-of-function clones. Larvae of the appropriate genotypes were heat shocked for 1 h at 37°C, at different larval stages. The clones were visualized in discs by either loss of GFP or β-Gal expression.

Immunostaining of embryos and discs

Discs were dissected in PBS and fixed in 4% paraformaldehyde for 20 min at room temperature. They were subsequently washed in PBS, blocked in blocking buffer (PBS, 0.3% Triton, 1% BSA), and incubated overnight with the primary antibody diluted in blocking buffer at 4°C. Washes were performed in blocking buffer, and the appropriate fluorescent secondary antibody was added for 1 h at room temperature. Following further washes in blocking buffer, the discs were mounted in Vectashield. Anti-FOXO antibody was kindly provided by Oscar Puig, anti-β-Gal (rabbit) and anti-caspase-3 were purchased from Cappel and from Cell Signalling, respectively. Images were taken in a laser MicroRadiance microscope (Bio-Rad) and subsequently processed using Adobe Photoshop. In situ hybridization was performed as described in (Arpiaza and Frasch, 1993), and embryos were mounted in Permount (Fisher Scientific). calderón antisense DIGoxigenin-labelled RNA probes were generated as described in (Tautz and Pfeifle, 1989) using the EST SD08136 (Berkeley Drosophila Genome Project).

Preparation of larval and adult cuticles

Adult flies were prepared by the standard methods for microscopic inspection. Soft parts were digested with 10% KOH, washed with alcohol and mounted in Euparal. Embryos were collected overnight and aged an additional 12 h. First instar larvae were dechorionated in commercial bleach for 3 min and the vitelline membrane was removed using heptano-methanol 1:1. After washes with 100% methanol and 0.1% Triton X-100, larvae were mounted in Hoyer’s lactic acid (1:1) and allowed to clear at 65°C for at least 24 h.

RESULTS AND DISCUSSION

calderón encodes an organic cation transporter expressed in embryonic and imaginal tissues

The LP1-Gal4 line was found in an enhancer trap screen performed in the adult fly (Calleja et al., 1996; Herranz and Morata, 2001). We selected this line for further analysis on the basis of its expression pattern in the developing embryo (see below). The insertion is located on the right arm of chromosome 3 polytene section 95F8, 422 bp upstream of the transcription start site of CG13610 (Fig. 1A). We analyzed CG13610 expression during embryonic and imaginal disc development. The expression pattern found in LP1-Gal4; UAS-lacz embryos and imaginal discs was similar to that of CG13610, as visualized by in situ hybridization (Fig. 1). From now onwards we refer to CG13610 as calderón (cald) and LP1-Gal4 as cald-Gal4. cald started to be expressed ubiquitously at the cellular blastoderm stage (Fig. 1C). At germ band extended stage, cald transcripts were found in a broad region of the dorsal side (Fig. 1F). As development proceeded, cald expression was restricted to amnioserosa cells and the central nervous system (Fig. 1B,G,H; a transverse section of the embryo at this stage showed an expression pattern that resembled the shape of a ‘calderón’, the Spanish name for the music symbol that increases the length of a music note). cald was ubiquitously expressed in the eye, leg, wing and haltere discs, although with regional modulation (Fig. 1I-N); its expression was stronger in the pouch region of the wing (Fig. 1L) and in the region posterior to the morphogenetic furrow in the eye (Fig. 1K,L). The antennal disc showed two rings of higher cald expression (Fig. 1K,L), while expression in the leg disc was greater in the distal domain (Fig. 1M,N).

CG13610 has been mapped cytologically to 95F8, encodes a 567-amino-acid-protein with 11 transmembrane domains, with highest homology with organic cation transporters of the major facilitator superfamily. Genes identified as putative homologs have been identified in Homo sapiens, M. musculus and G. Gallus, among others (Fig. 1O and data not shown). Mutations in the human homolog slc22a4 cause rheumatoid arthritis (Tokuhiro et al., 2003) and Crohn’s disease (Peltekova et al., 2004), probably because of defects in the transport of organic metabolites.

Embryonic and imaginal requirement of calderón

The original cald-Gal4 insertion is viable without any overt phenotype. To perform a functional analysis of cald, we induced mutations by mobilization of the P-element. We isolated three embryonic lethal alleles (caldR106, caldR107, caldR165), and one homozygous viable allele (caldR165). Sequence analysis revealed that caldR106 and caldR107 bear truncated P-elements of 2.7 and 9 Kb, respectively, but 11 bp further downstream from the cald-Gal4 original insertion site. caldR106 has a P-element fragment of only 50 bp located at the same insertion point as the two previous lethal alleles. We were unable to characterize the caldR165 allele. The lethal alleles caldR165, caldR106 and caldR107 greatly reduce CG13610 mRNA expression (compare Fig. 2E with F, and data not shown). Another independent P-element insertion, EP1072, located further upstream of the original cald-Gal4 insertion, is embryonic lethal, strongly reduces the levels of CG13610 mRNA expression and drives the expression of calderón in a GAL4-dependent way (Fig. 2G,H and data not shown). Embryos homozygous for the caldR107, caldR165, caldR106 and caldR107 mutations showed the characteristic U-shape phenotype of embryos unable to retract the germ band, probably because of abnormal development of the amnioserosa [compare Fig. 2B,C with 2A (Frank and Rushlow, 1996)]. Embryo-mutants for InR present the same phenotype (Fig. 7F) (Fernandez et al., 1995).
Development of flies homozygous for the viable allele calderón was markedly delayed during larval and pupal development (Fig. 3A). Adult flies were smaller than wild-type animals (fly length ratio of calderón/wild-type animals was 0.9±0.04; Fig. 3B,E). Mutant wings and eyes were also smaller (eye size ratio of calderón/wild-type animals was 0.8±0.05; wing size ratio of calderón/wild-type animals was 0.85±0.04; Fig. 3C-E). Cell density was increased in the absence of calderón activity, indicating that cell size but not cell number was affected (wing cell density ratio of calderón/wild-type animals was 1.3±0.16; Fig. 3E).
Fortuitously, the lethal excision lines \textit{cald}\textsuperscript{R106} and \textit{cald}\textsuperscript{R107} (but not \textit{cald}\textsuperscript{R165}) leave \textit{GAL4} and the cis-regulatory regions unaffected, so that the mutants express \textit{GAL4} in the normal pattern of \textit{calderón} (Fig. 2D). Interestingly, these two alleles complemented the lethal allele \textit{cald}\textsuperscript{EP1072}, probably as a result of the capacity of \textit{GAL4} expressed in the normal pattern of \textit{cald} (in \textit{cald}\textsuperscript{R106} and \textit{cald}\textsuperscript{R107} \textit{P} element insertions) to drive the expression of CG13610 under the control of the \textit{GAL4}-binding sites (in the \textit{cald}\textsuperscript{EP1072} insertion, Fig. 2H). It is well known the \textit{GAL4}/\textit{UAS} system is cold sensitive, being most effective at 25°C. \textit{cald}\textsuperscript{R106}/\textit{cald}\textsuperscript{EP1072} adult flies did not show any growth defect when raised at 25°C (Fig. 3F,G). When raised at 18°C, flies were smaller (Fig. 3F,G), resembling the growth phenotype of the \textit{cald} viable allele \textit{cald}\textsuperscript{R161}. Taken together, these results indicate that the embryonic phenotype and the growth defects of the \textit{cald} mutants are caused by the absence of CG13610 expression.
Cells lacking calderón show reduced proliferation and are eliminated by cell competition

To study cald requirement during imaginal development we induced clones of cells mutant for cald. Lethal allele caldR107 was used in this analysis. The frequencies and sizes of cald mutant clones and their twin clones were similar 24 h after induction (Fig. 4A), but after 48 h cald mutant clones contained significantly fewer cells and were recovered at a lower frequency (Fig. 4B). At 72 h after induction, mutant clones were not recovered (Fig. 4C), indicating that cald homozygous cells were eliminated from the disc epithelium. Clones expressing a cald transgene (Fig. 4D) under the control of the tubulin promoter [using the MARCM technique (Lee and Luo, 1999)] were recovered 72 h after induction, demonstrating that these defects are caused by loss of cald activity.

All these results indicate that cald mutant cells can perform several rounds of divisions but at a lower rate than control cells. Thus, their elimination might be due to cell competition of slow-dividing cells (Morata and Ripoll, 1975; Moreno et al., 2002). Indeed, caldR107 clones showed increased activity of the effector caspase drice (Fraser et al., 1997), a cysteine protease that degrades the cellular substrates and causes cell death (Fig. 4E,E'). This apoptosis is associated with the upregulation of brinker (Moreno et al., 2002). caldR107 clones also exhibit upregulation of the expression of this gene (Fig. 4F,F').

To circumvent the problem caused by slow proliferation and apoptosis of cald mutant cells, and to verify that these cells are lost by cell competition, we used the Minute technique (Fig. 5A) (Morata and Ripoll, 1975) to increase their proliferation rate. The Minute mutations are defective in ribosomal proteins, and in heterozygous conditions produce a developmental delay caused by their low division rate. cald Minute+ clones growing in a Minute+/background lost the retarding Minute condition and compensated for the slowdown of proliferation. Under these conditions, cald Minute+ clones were recovered (Fig. 5C,E,G). However, they were smaller than wild-type control clones (compare Fig. 5F with G). The size ratio of calderón/wild-type clones was 0.13±0.1, and the density ratio was 1.24±0.29. Thus, the average number of cells per clone and the cell size were reduced, indicating that the loss of cald activity makes cells grow and proliferate at slower rates.

The Minute homozygous condition is cell lethal. Under normal circumstances, Minute homozygous cells resulting from mitotic recombination (Fig. 5A) were not recovered 48 h after induction (Fig. 5D). When cald Minute+ mutant clones were analyzed, the frequency of recovered Minute+ clones was much higher than expected (Fig. 5C,E,G-I). These results indicate that Minute+ homozygous clones are eliminated from the disc epithelium as a result of cell competition and either the loss of cald activity in neighbouring cells (i.e. the mutant clones), or the cell-autonomous increase in cald activity in the Minute+ homozygous cells reduces cell competition and allows Minute+ cells to survive longer.

The examination of cald mutant clones in the wing epithelium and adult cuticle revealed that mutant cells were smaller (Fig. 6). caldR107 Minute+ cells were smaller than wild-type cells in the wing epithelium (Fig. 6A,B), and differentiated thinner and shorter bristles at the adult wing margin (Fig. 6E,F) and in the notum (Fig. 6C,D). These results indicate that cald has a cell-autonomous effect on cell size.

Growth stimulation by the InR pathway requires calderón

The cald embryonic phenotype and the cell-autonomous effect on the rate of cell division are reminiscent of mutations in components of the InsR signalling pathway (Coelho and Leevers, 2000). It is conceivable that cald is a new component or target of the InR pathway. In response to ligand binding, the InsR phosphorylates the Insulin Receptor Substrate (IRS) proteins (encoded by chico in Drosophila) (Böhni et al., 1999), thereby activating the class I PI 3-kinase (PI3K), which in turn increases the levels of the second messenger phosphatidylinositol 3,4,5-triphosphate (PIP3) at the cell membrane (Fig. 8). The serine threonine protein kinase Akt appears to be the major critical target of PIP3 signalling in Drosophila. Two signalling branches downstream of Akt have been identified. One leads to activation of TOR and S6K through Rheb kinase activity. The Drosophila Rheb functions in the InR pathway downstream of Tsc1-Tsc2, and its overexpression causes activation of this pathway, leading to increased cell proliferation (Garami et al., 2003; Zhang et al., 2003). To determine whether cald is required for Rheb signalling, we used the MARCM technique to examine the growth properties of clones of cells that lack cald and overexpress Rheb (Lee and Luo, 1999). Rheb overexpression did not induce growth in cald mutant cells and these clones were lost 72 h after induction (not shown). We therefore conclude that cald functions downstream of or in parallel to Rheb signalling.
A major effector of Rheb function is S6K (Stocker et al., 2003). Genetic evidence has established that insulin exerts many of its cellular effects by triggering the activation of S6K (p70 ribosomal S6 kinase). Full activation of dS6K requires two distinct signals, one in response to growth factors and another from a nutrient sensing pathway, and thus provides a mechanism whereby individual cells can coordinate their response to growth factors with nutrient availability (Zhang et al., 2003). To determine whether cald is involved in nutrient sensing, we examined the growth properties of clones of cells that lack cald and overexpress dS6K. As with Rheb, cells overexpressing dS6K but lacking cald activity were lost by 72 h after clone induction (not shown). We conclude that cald is not involved in nutrient sensing and is epistatic to S6K. Therefore, cald functions downstream of or in parallel to S6K signalling.

Fig. 5. The role of calderón activity in Minute mediated cell competition. (A) Diagram depicting the induction of Minute+ cald mutant clones (upper cell) and their Minute− cald+ twins (lower cell) after a mitotic recombination event. (B-G,I) Clones are marked by the absence of the β-Gal marker (grey). Twins are marked by the presence of two copies of the β-Gal marker (white). (B,D,F) Minute+ control clones visualized 24 (B), 48 (D) and 72 (F) h after induction. Note all twin Minute+ homozygous clones have already disappeared 48 h after induction. (C,E,G,I) cald Minute+ clones visualized 24 (C), 48 (E) and 72 (G,I) h after induction. Some twins (Minute+ homozygous and wild type for cald function) are recovered even 72 h after induction (magnified in I). (H) Graph plotting the ratio of Minute+/Minute− control clones (in black) and the ratio of Minute+/Minute− cald mutant clones (in red). 24 h after clone induction: number of Minute+ clones: 522; number of Minute− cald clones: 253. 48 h after clone induction: number of Minute+ clones: 507; number of Minute− cald clones: 187. 72 h after clone induction: number of Minute+ clones: 58; number of Minute− cald clones: 81.

Fig. 6. calderón clonal phenotypes. (A,B) cald mutant cells are small in imaginal discs. Minute+ cald187 mutant clones marked by the absence of the GFP marker (green) in the wing imaginal disc. Cell membranes are delineated by Phaloidin (red in A) and cell nuclei are labelled by DAPI (blue in B). dFOXO (red in B) remains cytoplasmic in cald clones. The white trace outlines the mutant clone in B. (C-F) cald mutant cells are small in the adult. Clones of Minute+ cald mutant cells marked by the absence of the P(yellow+) rescue construct in the notum (C,D), and in the wing margin (E,F). Each hair-like structure is a trichome emanating from a single epithelial cell. Note the reduced size and increased density of cald cells (red arrow) compared with surrounding yellow+ ones (black arrowhead).
A second pathway downstream of Akt has been identified. The Forkhead-related transcription factor dFOXO is phosphorylated by Akt upon insulin signalling, leading to cytoplasmic retention and inhibition of its transcriptional activity. Upon reduced insulin signalling, dFOXO becomes dephosphorylated and accumulates in the nucleus, where it regulates the transcription of a number of target genes, thereby leading to cell death or cell cycle arrest (Junger et al., 2003; Kramer et al., 2003; Puig et al., 2003). To determine whether cald is required for dFOXO inhibition, we examined the subcellular location of dFOXO in clones of cald mutant cells, and found that dFOXO remains cytoplasmic in these clones (Fig. 6B). We conclude that Akt inhibits dFOXO in the absence of cald function. These results indicate that cald is not required for dFOXO inhibition.

**InR pathway regulates calderón expression**

PI3K and TOR-mediated signals exert some of their growth effects at the transcriptional level (reviewed in Neufeld, 2003). TOR controls expression of a broad group of genes with roles in protein, lipid and nucleic acid metabolism (Beck and Hall, 1999; Cardenas et al., 1999). In addition, both TOR and PI3K affect the expression and activity of the C/EBP family of transcriptional regulators, which regulates the expression of a number of genes in response to nutrients (Entingh et al., 2001; Roessler, 2001). Thus, given that the InR signalling pathway controls the expression of a diverse spectrum of metabolism-related genes, we examined whether this pathway activates cald transcription. To address this issue, cald expression was first analyzed in the embryo following expression of either S6K or a dominant-negative form of the Drosophila PI3K (PI3K92E-Dp110<sup>954A</sup>). Expression of S6K in the patched or Ubx domains induced ectopic cald expression (Fig. 7C,E, compare with 7A). When a dominant-negative version of PI3K92E-Dp110 (PI3K92E-Dp110<sup>954A</sup>) was expressed in amnioserosa cells under the control of the Kr-GAL4 driver, cald expression was reduced in these cells (compare Fig. 7B with D, red arrows). Furthermore, these embryos did not resect the germ band (Fig. 7F), as expected for an embryo that lacks cald expression in the amnioserosa (Fig. 2B,C). When PI3K92E-Dp110<sup>954A</sup> was expressed in the dorsal side of the wing imaginal disc under the control of the MS1096-GAL4 driver, cald expression was reduced (compare Fig. 7G with H). Expression of S6K in the patched domain did not increase cald expression in the wing disc, suggesting that the expression levels of this gene are saturated in imaginal cells (data not shown). Consistent with this, over-expression of cald in clones of cells did not cause any overt phenotype in terms of growth, cell size or cell competition (data not shown). Taken together, the data indicate that cald expression is regulated by the InR pathway in the embryo and in imaginal cells.

**Concluding remarks**

Genetic studies have revealed a key role for InR signalling in coordinating growth and other nutrition-regulated functions in flies. Although the distinct elements of this pathway have been well described, the downstream elements responsible for this task remain to be fully elucidated. calderón, a gene encoding for an Organic Cation Transporter, is regulated at a transcriptional level by the PI3- kinase/TOR pathway (Fig. 8). cald mutant flies are smaller than wild-type ones and show a developmental delay. Clones of cells mutant for cald divide more slowly, are smaller than wild-type cells and are eliminated by cell competition. However, when cald clones have a proliferative advantage in a Minute heterozygous background, they are not eliminated and are able to differentiate normal adult structures, although they are smaller than wild-type ones. Since cald vertebrate orthologs are involved in carrying organic substrates across the plasma membrane (Grundemann et al., 2005), we propose that reduced cald activity in proliferating cells impairs competition for organic substrates available in the extracellular media. This impairment may again represent (Moreno et al., 2002) another mechanism by which weaker cells are removed from a growing population, and might serve to regulate cell number and optimise tissue fitness and hence organ function.
cold activity is required for PI3-kinase/TOR function in inducing growth: up-regulation of this pathway in a cold mutant background does not affect growth. Furthermore, cold expression is dependent on PI3-kinase activity, as embryos overexpressing S6K show increased cold levels, whereas expression of a dominant negative form of PI3K (PI3K92E-Dp110^{BDS4A}) reduces them. Thus, we propose that cold responds to InR activity levels and that it is required, in a cell-autonomous way, for cell growth and proliferation. We hypothesize that the growth of imaginal cells is then controlled by two distinct, but coordinated, nutrient-sensing pathways. We summarize and feedback regulation of the insulin receptor pathway. Cell 96, 563-573.

References


