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A Genetic Screen for Dominant Modifiers of a Small-Wing Phenotype in *Drosophila melanogaster* Identifies Proteins Involved in Splicing and Translation

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ABSTRACT

Studies in the fly, *Drosophila melanogaster*, have revealed that several signaling pathways are important for the regulation of growth. Among these, the insulin receptor/phosphoinositide 3-kinase (PI3K) pathway is remarkable in that it affects growth and final size without disturbing pattern formation. We have used a small-wing phenotype, generated by misexpression of kinase-dead PI3K, to screen for novel mutations that specifically disrupt organ growth *in vivo*. We identified several complementation groups that dominantly enhance this small-wing phenotype. Meiotic recombination in conjunction with visible markers and single-nucleotide polymorphisms (SNPs) was used to map five enhancers to single genes. Two of these, *nucampholin* and *prp8*, encode pre-mRNA splicing factors. The three other enhancers encode factors required for mRNA translation: *pixie* encodes the Drosophila ortholog of yeast RLI1, and *RpL5* and *RpL38* encode proteins of the large ribosomal subunit. Interestingly, mutations in several other ribosomal protein-encoding genes also enhance the small-wing phenotype used in the original screen. Our work has therefore identified mutations in five previously uncharacterized Drosophila genes and provides *in vivo* evidence that normal organ growth requires optimal regulation of both pre-mRNA splicing and mRNA translation.

NORMAL biological development and homeostasis require tight control of growth at the level of individual cells, organs, and the whole organism. For example, unregulated cellular proliferation may result in too few or too many cells, leading to inappropriately sized, nonfunctional organs, which in turn can result in a variety of pathological conditions. Significantly, individuals of the same species raised in a given environmental niche grow to similar final sizes. This means that growth is controlled, at least to some extent, genetically. Such "growth genes" might encode key components or effectors of discrete growth-regulatory signaling pathways.

In recent years, the fruit fly, *Drosophila melanogaster*, has been used as a model organism to investigate the genetic basis of growth control (reviewed in Edgar and Nijhout 2004). Most of these studies have focused on the growth of the larval imaginal discs. Imaginal discs are epithelial structures that undergo massive growth during the ~4 days of larval life. The size of the disc at the end of the larval period largely determines the size of the adult appendage (eye, wing, etc.) into which it ultimately develops (reviewed in Johnston and Gallant 2002).

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Key regulators of Drosophila imaginal disc growth have been discovered through three main approaches. First, classical genetic research has produced many mutant strains that exhibit growth phenotypes. For example, *Minute* mutants, which correspond to at least 50 different genetic loci, have a slower growth rate and sometimes an altered adult size (Lambertsson 1998). These phenotypes are thought to be the result of a reduced capacity for protein synthesis. Indeed, several *Minute* mutations have been demonstrated to disrupt genes that encode ribosomal proteins (RPs) (Lambertsson 1998).

A second way in which Drosophila growth regulators have been discovered is through the study of proteins or signaling pathways whose mammalian orthologs have been implicated in cell proliferation and/or growth. An example of this approach is the characterization of the insulin receptor/phosphoinositide 3-kinase (InR/PI3K) pathway (reviewed in Leevers and Hafen 2004). For example, clonal loss of *Dp110*, which encodes the catalytic subunit of the Drosophila class 1A PI3K, reduces cell size and clonal growth in imaginal discs (Weinkove *et al.* 1999). Conversely, overexpression of *Dp110* increases cell size, promotes cell cycle progression, and causes tissue overgrowth (Leevers *et al.* 1996). Similar results have been obtained through modulating the activity of other components of the pathway

(Leevers and Hafen 2004). Significantly, final organ/body size is altered without affecting pattern or shape in all these experiments, thereby demonstrating that the InR/PI3K pathway regulates growth and determines final size *per se* rather than as a secondary result of effects on patterning. The exact mechanism through which InR/PI3K signaling results in growth is poorly understood, but is likely to involve targets of Akt and the Foxo transcription factor and the upregulation of protein synthesis (Leevers and Hafen 2004).

Drosophila Ras (Prober and Edgar 2000), Myc (Johnston *et al.* 1999; de la Cova *et al.* 2004), Tor (Zhang *et al.* 2000), and the Cyclin D–Cyclin-dependent kinase 4 complex (CycD–Cdk4) (Datar *et al.* 2000; Meyer *et al.* 2000) have also been demonstrated to regulate fruit fly growth subsequent to their initial characterization in other species. In response to high levels of nutrients and/or growth factors, Tor stimulates S6 kinase and the translation initiation factor eIF-4E and is thought to promote growth, at least in part, through upregulation of protein synthesis (reviewed in Neufeld 2004). CycD–Cdk4 is remarkable in that it promotes cellular growth and cell cycle progression in a coordinated manner and can therefore increase overall organ size (Datar *et al.* 2000; Meyer *et al.* 2000). This is in stark contrast to other components of the Drosophila cell cycle machinery, which alter cell division rates without directly affecting cell growth (Neufeld *et al.* 1998).

The third major way in which growth regulators have been discovered in Drosophila is through various ingenious genetic screens. Screening has proved to be a powerful and relatively unbiased approach to identify both characterized genes that were not previously known to have a growth-regulatory role and completely novel genes. For example, some of the earliest investigations in this field uncovered zygotic mutations that cause overgrowth of the larval imaginal discs (reviewed in Bryant *et al.* 1993 and Watson *et al.* 1994). A number of "tumor suppressor" genes, including *fat* and *discs large 1*, were discovered in this way. Taking the opposite approach, Galloni and Edgar screened homozygous mutant larvae for abnormally small sizes and developmental delay and so identified mutations in the genes encoding translation initiation factor eIF-4a and the mitochondrial RP, mRpS15 (Galloni and Edgar 1999).

Several laboratories have conducted clonal growth screens to circumvent the early lethality that often results from zygotic mutation of growth genes. In most of these screens, clones that are homozygous for random mutations were induced in the early eye imaginal disc and the adult eye was subsequently examined for over- or undergrowth. Genes identified in this manner include *Pten* (Gao *et al.* 2000), *Tsc1* (Gao and Pan 2001; Potter *et al.* 2001; Tapon *et al.* 2001), *Tsc2* (Ito and Rubin 1999), *Rheb* (Stocker *et al.* 2003), *Tor* (Oldham *et al.* 2000), *warts* (Justice *et al.* 1995; Xu *et al.* 1995), *salvador* (Kango-Singh *et al.* 2002; Tapon *et al.* 2002), and *hippo* (Harvey *et al.* 2003; Jia *et al.* 2003; Udan *et al.* 2003; Wu *et al.* 2003). Tor, Rheb, and the Tsc1–Tsc2 complex are all core components of the Tor signaling pathway (reviewed in Leevers and Hafen 2004). Interestingly, Tsc1–Tsc2 can be inhibited by InR/PI3K signaling, thus forming a link between the InR/PI3K and Tor pathways (reviewed in Marygold and Leevers 2002). Warts, Hippo, and Salvador form a complex that restricts growth by promoting both cell cycle exit and apoptosis (reviewed in Ryoo and Steller 2003).

As an alternative approach, other groups have screened for growth defects induced by overexpressing random genes in organs such as the developing eye or wing. Genes identified in this way include *Rheb* (Patel *et al.* 2003; Saucedo *et al.* 2003; Stocker *et al.* 2003), *bantam* (Hipfner *et al.* 2002; Raisin *et al.* 2003), and *slimfast* (Colombani *et al.* 2003). Growth regulation through the *bantam* and *slimfast* gene products occurs through very different mechanisms. *bantam* encodes a micro-RNA that promotes growth by simultaneously stimulating proliferation and preventing apoptosis, probably through suppressing the translation of specific mRNA targets (Brennecke *et al.* 2003). Slimfast is an amino acid transporter, the analysis of which revealed that the larval fat body acts as a nutrient sensor that can regulate organismal growth through a systemic mechanism (reviewed in Bradley and Leevers 2003).

Yet another screening method has been to search for genetic modifiers of growth-sensitized phenotypes. This more subtle approach can uncover important growth genes that might otherwise be missed. For example, a screen for modifiers of the big eye resulting from CycD–Cdk4 overexpression identified mutations in the genes encoding Hif-1 prolyl hydroxylase (Hph) (Frei and Edgar 2004) and the mitochondrial RP mRpL12 (Frei *et al.* 2005). In a different screen, overexpression of either *scylla* (*scy*) or *charybdis* (*char*) was found to suppress the big-eye phenotype achieved by coexpression of Akt1 and Pdk1 (Reiling and Hafen 2004). Interestingly, Hph, mRpL12, Scy, and Char are all involved in linking oxygen sensing to growth control.

It is clear that a wide variety of Drosophila growth genes have been identified through loss-of-function, overexpression, and modifier screens conducted in whole larvae, specific adult organs, or discrete clones of cells. We wished to identify additional factors that affect organ growth without altering pattern. To do this, we have screened for randomly induced mutations that dominantly enhance or suppress a small-wing phenotype obtained by expression of kinase-dead PI3K. Such a screen may be expected to identify factors that are limiting for organ growth *in vivo*.

MATERIALS AND METHODS

Fly stocks: Fly strains are described at FlyBase (http://flybase.bio.indiana.edu/) and are available from the

Bloomington *Drosophila* Stock Center when a reference is not given.

Basic strains: w^{1118}*-iso*, Oregon-R, or *y w* was used as a control strain. *MS1096-GAL4* (Capdevila and Guerrero 1994), *UAS-Dp110*^{KD} (Leevers *et al.* 1996), *UAS-Argos.M30-102-1* (A. Michelson, unpublished data), *UAS-Argos.III* (MacDougall *et al.* 2004), *P[neoFRT]82B ry*^{506}, and *P[hsFLP]1; P[neoFRT]82B P[Ubi-GFP*^{S65T}*nls]3R* were the basic strains used. The *MS1096-GAL4 UAS-Dp110*^{KD}, *MS1096-GAL4 UAS-Argos.M30-102-1*, and *MS1096-GAL4; UAS-Argos.III* stocks were made in our laboratory. Mapping strains are described in the mapping section below.

InR/PI3K pathway mutants: InR/PI3K pathway mutants were *InR*^{31} (Fernandez *et al.* 1995), *InR*^{339} (Brogiolo *et al.* 2001), *chico*^{1} and *chico*^{2} (Bohni *et al.* 1999), *Dp110*^{A} *P[gH]* and *Dp110*^{B} *P[gH]* and *p60*^{A}; *P[gR10]* and *p60*^{B}; *P[gR10]* (Weinkove *et al.* 1999), *Pten*^{dj189} (Gao *et al.* 2000), *Pten*^{3} (Goberdhan *et al.* 1999), *Akt1*^{6M4} (H. Stocker and E. Hafen, unpublished data), *Akt1*^{1} (Staveley *et al.* 1998), *foxo*^{21} and *foxo*^{25} (Junger *et al.* 2003), *Tsc1*^{Q87X} and *Tsc1*^{R453X} (Tapon *et al.* 2001), *Rheb*^{2D1} and *Rheb*^{7A1} (Stocker *et al.* 2003), and *Tor*$^{\Delta P}$ and *Tor*^{2L19} (Oldham *et al.* 2000).

Other mutants: Other mutants were *ncm*^{SH0931} (Oh *et al.* 2003), *eIF-4a*^{1006} and *eIF-4a*^{1069} (Galloni and Edgar 1999), *eIF-3p40*^{k09003}, *eIF-4E*^{07238}, *dp*^{ov1}, *dp*^{lv1}, and *dp*^{olvR}. RP mutants are listed in Table 2.

Deficiencies: The X chromosome deficiency kit (*c.* 1998) from the Bloomington *Drosophila* Stock Center was used in the pilot screen. The interacting deficiencies shown in Figure 1E are: S-1, *Df(1)BA1*; E-1, *Df(1)N-8* and *Df(1)dm75e19*; E-2, *Df(1)G4e*^{L} *H24i*^{R}; E-3, *Df(1)KA14*; E-4, *Df(1)KA7* and *Df(1)HA85*; E-5, *Df(1)N105* and *Df(1)JA26*; E-6, *Df(1)C246*; E-7, *Df(1)sd72b*; E-8, *Df(1)N19*; E-9, *Df(1)JA27*; and E-10, *Df(1)HF396* and *Df(1)A209*. (A full list of the deficiencies used in the pilot screen is available upon request.) *pix* is deleted/disrupted in *Df(3L) Scf-R6* and *Df(3L)Scf-R11*. *prp8* is deleted/disrupted in *Df(2R)BSC40*, but is not deleted/disrupted in *Df(2R)CB21*. *ncm* is deleted/disrupted in *Df(2L)TW137*, *Df(2L)M36F-S5*, *Df(2L)M36F-S6*, and *Df(2L)M36F2* [previously called *M(2) 36F*^{2}] but not in *Df(2L)T317*, *Df(2L)H20*, *Df(2L)TW50*, *Df(2L)OD15*, *Df(2L)TW3*, or *Df(2L)VA16*. [Additional data on deficiencies in the vicinity of *ncm* have been deposited at FlyBase (http://flybase.bio.indiana.edu/).] Deficiencies used in the mapping of *RpL38* and *RpL5* are described in Marygold *et al.* (2005).

New dumpy alleles: E-2f^{1}, *E-2f*^{2}, and *E-2f*^{3} are lethal in *trans* with each other and also in *trans* with a *CyO* or *SM6a* balancer chromosome. Both *CyO* and *SM6a* contain a lethal mutation in *dumpy* (*dp*) (Lindsley and Zimm 1992). *E-2f*^{1}, *E-2f*^{2}, and *E-2f*^{3} were subsequently found to be lethal in *trans* with independently derived lethal (*l*) *dp* mutations (*dp*^{lv1} and *dp*^{olvR}) and show the characteristic oblique (*o*) wing and/or thoracic vortex (*v*) phenotypes in *trans* with the viable *dp*^{ov1} mutation. We have named these three new *dp* alleles as *dp*$^{2f1\text{-}olv}$, *dp*$^{2f2\text{-}lv}$, and *dp*$^{2f3\text{-}olv}$, according to the nomenclature of Carlson (1959).

Mutagenesis: *FM7/Y* males were fed with 25 mm ethyl methanesulfonate (EMS; Fluka, Buchs, Switzerland) in 10% sucrose solution as described (Newsome *et al.* 2000) and mated *en masse* with *MS1096>Dp110*^{KD} virgin females.

Mutant phenotype analyses: Our standard fly culture conditions were to cross ~10 virgin females to ~5 males in fresh vials containing standard food; females were allowed to lay eggs for 2 days before transferring the adults to a new tube. Crosses were maintained at 25°. Where necessary, the desired F_1 progeny were identified by selecting against dominant markers on balancer chromosomes.

Crosses to assess the degree of dominant modification of the *MS1096>Dp110*^{KD} and *MS1096>Aos* wing phenotypes by *E-Dp110*^{KD} mutations were performed at least three times and two times, respectively. *E-Dp110*^{KD} mutations were outcrossed to a wild-type strain under our standard culture conditions to assess dominant effects on wing size; the area of *E-Dp110*^{KD}/+ female wings was then compared to that of +/+ female wings. At least two different alleles of InR/PI3K pathway mutants were used in crosses to *MS1096>Dp110*^{KD}. Appropriate controls were performed for each test cross under identical culture conditions. Approximately 20 wings were measured for control genotypes and 10 wings were measured for test genotypes. Male and female wings were measured separately. Adult wings were processed and their areas measured as described (Marygold *et al.* 2005). Statistical analyses were performed using Microsoft Excel: *P*-values were calculated using a two-tailed Student's *t*-test assuming equal variances.

Dominant Minute bristle phenotypes of *E-Dp110*^{KD} mutants were originally assessed in the stocks; where evident, this was confirmed by outcrossing *E-Dp110*^{KD} mutants to a wild-type strain under uncrowded culture conditions and examining F_1 progeny. The RP mutants listed in Table 2 were simultaneously outcrossed to the w^{1118}*-iso* strain under uncrowded culture conditions; F_1 progeny were ranked according to their dominant developmental delay and bristle phenotypes were compared to those of w^{1118}*-iso* flies raised under identical conditions.

For clonal analyses, *lep*^{3c5} and *lep*^{3c6} were recombined onto the *P[neoFRT]82B* chromosome arm and crossed to *P[hsFLP]1; P[neoFRT]82B P[Ubi-GFP*^{S65T}*nls]3R*. Clones were induced at mid-third instar by a 34° heat shock for 10 min. Adult eyes were processed as described (Tomlinson and Ready 1987).

Mapping of mutations: Mapping was performed for the third and second chromosome enhancer mutations and the second chromosome suppressor mutations using methods described separately below. In each case, the mapping chromosome itself does not dominantly affect wing size or morphology (data not shown).

Third chromosome E-Dp110^{KD} *mutations:* To roughly map *lep*^{3c5} and *lep*^{3c6}, recombination was allowed between the mutant chromosomes and a *st*^{1} *Sb*$^{sbd\text{-}1}$ *e*^{s} *ro*^{1} *ca*^{1} mapping chromosome in the presence of *MS1096>Dp110*^{KD}. Approximately 250 *lep*^{3c5} F_1 recombinants and ~80 *lep*^{3c6} F_1 recombinants were analyzed for the presence or absence of the enhancer mutation. To roughly map the other five *E-3c* mutations, recombination was allowed between the mutant chromosome and a *ru*^{1} *h*^{1} *st*^{1} *ry*^{506} *e*^{1} mapping chromosome. Initially, ~50 individual male recombinants were crossed to *MS1096>Dp110*^{KD} female flies to test for the presence or absence of the enhancer mutation to map the mutation between two of the visible markers. In a second round of recombination, ~100 males in which recombination had occurred between the two markers of interest were tested for the presence or absence of the enhancer mutation. The percentage of frequency of recombination between the *E-3c* mutation and the individual markers was used to calculate recombination distance in each case.

For the fine mapping of *pix*, recombinants were generated between the *pix*^{3c3} chromosome and a chromosome containing two closely spaced *P[w*$^{+}$*]* elements [*l(3)s2383*^{s2383}*-l(3)j2B9*^{j2B9}, a recombinant chromosome made in our lab] that flanked the region of interest (ROI) defined by the initial meiotic recombination and deficiency mapping. Whereas the *P[w*$^{+}$*]-P[w*$^{+}$*]* parental flies have a relatively dark eye color because of having two *[w*$^{+}$*]* transgenes, recombination events between the *P[w*$^{+}$*]* elements generate recombinant flies with a single *P[w*$^{+}$*]* element and a visibly paler eye color. Thus, informative recombinants with DNA breaks within the ROI were specifically selected. These recombinants were crossed to the *pix*^{3c2} allele and to *MS1096>Dp110*^{KD} to test for the presence of the *pix*^{3c3} mutation and were analyzed by PCR to identify which

P[w⁺] element was present. Single-nucleotide polymorphisms (SNPs) in the ROI were identified (supplementary Table S2 at http://www.genetics.org/supplemental/) and used to genotype ~200 recombinant chromosomes.

Second chromosome E-Dp110^{KD} mutations: E-Dp110^{KD} chromosomes were allowed to recombine with an isogenized *wg^{Sp-1} Bl^{1} L^{rm} Bc^{1} Pu^{2}* mapping chromosome. Only *wg^{Sp-1}*, *Bl^{1}*, and *L^{rm}* were used for mapping and we refer to this chromosome as "*Wg-Bl-L*." In the first stage of mapping using visible markers, ~40 recombinant males that contained a chromosomal break between the two terminal markers (*Wg* and *L*) were isolated; additional recombinant males were stored at 18°. The initial 40 recombinants were tested for the presence or absence of the *E-Dp110^{KD}* mutation by testing for enhancement of *MS1096>Dp110^{KD}* and/or noncomplementation of the other mutant allele and then mapping the mutation either between two of the markers or distal to one of the terminal markers. A further ~100 recombinants (from those stored at 18°) that contained the appropriate chromosomal break were then selected, and the presence or absence of the *E-Dp110^{KD}* mutation was again determined. The percentage of frequency of recombination between the *E-Dp110^{KD}* mutation and each adjacent marker in these 100+ recombinants was used to determine genetic linkage and so further narrow the ROI.

In the second stage of finer-scale mapping, SNPs between the *E-Dp110^{KD}* chromosome and the *Wg-Bl-L* chromosome were sought within the defined ROI. Initially, SNPs spaced widely in the ROI were found and used to genotype DNA prepared from all 100+ recombinants. More closely spaced SNPs were then used to genotype the progressively fewer informative recombinants remaining until either recombinants or SNPs were exhausted. Further details of this mapping strategy and a crossing scheme can be found in Martin *et al.* (2001). Second chromosome SNPs are described in supplementary Table S1 (http://www.genetics.org/supplemental/).

S-Dp110^{KD}: To map *S-2a*, recombination was allowed between the *S-2a^{2}* chromosome and the *Wg-Bl-L* mapping chromosome in the presence of *MS1096>Dp110^{KD}*. Approximately 250 *S-2a^{2}* F_1 recombinants were analyzed for the presence or absence of the suppressor mutation: *S-2a^{2}* was thus mapped to 54 cM, which is close to the *apterous* gene at 55 cM. The following observations strongly suggest that *S-2a* corresponds to *apterous*. First, *Df(2R)nap1* (41D2–E1; 42B1–3) deletes *apterous* (41F8), is semilethal in *trans* with *S-2a^{1}*, and weakly dominantly suppresses the *MS1096>Dp110^{KD}* small wing. Second, *S-2a^{1}/Df(2R)nap1* escapers and *S-2a trans*-heterozygous escapers lack wings, similar to certain *apterous* mutants. Finally, *S-2a^{1}* fails to complement two previously described *apterous* mutations (*ap^{4}* and *ap^{rK568}*) with escapers again showing a no-wing phenotype.

Molecular biology techniques: Isolation of genomic DNA from flies, primer design, PCRs, DNA sequencing, and sequence analysis were performed as described (Marygold *et al.* 2005). Crude DNA preparations from recombinant flies were made by crushing one to two prefrozen flies in 50 μl buffer [10 mm Tris-HCl (pH 8.2), 1 mm EDTA, 25 mm NaCl, 0.01 mg Proteinase K] in 0.2-ml PCR tubes or 96 well plates and incubating at 37° for 25 min and then at 95° for 5 min (to inactivate the Proteinase K); 2 μl of these crude preparations was used in a 25-μl PCR. Primers and specific PCR conditions used to amplify SNP regions, *ncm*, *prp8*, *pix*, and other candidate genes are available upon request; those used to amplify *RpL38* and *RpL5* are described in Marygold *et al.* (2005).

For single-strand conformation polymorphism (SSCP) detection, either Phast gels with the Phast electrophoresis system (Pharmacia Biotech, Piscataway, NJ) or GeneGel Excel kits (Amersham Biosciences, Little Chalfont, England) with the GenePhor electrophoresis system (Pharmacia Biotech) were used; PCR products were prepared according to the manufacturer's instructions and electrophoresis was performed at 10°. For restriction fragment length polymorphism (RFLP) detection, 10 μl of PCR product was digested in a total volume of 20 μl and incubated according to the instructions provided by the supplier [New England Biolabs (Beverly, MA) or Roche (Indianapolis)]; digested DNA was then subjected to electrophoresis on 1% agarose gels.

SNP identification and detection: In a few cases, previously reported SNPs (Hoskins *et al.* 2001) were tested for dimorphism between the mapping and *E-Dp110^{KD}* chromosomes and used for genotyping recombinant chromosomes. New SNPs were detected in PCR-amplified genomic DNA by sequencing ~1-kb regions or by performing SSCP analysis of 200- to 300-bp regions and then identifying bases/regions dimorphic between the mapping and *E-Dp110^{KD}* chromosomes. See supplementary Tables S1 and S2 (http://www.genetics.org/supplemental/) for a full listing of all SNPs identified. SNPs between the mutagenized second chromosome and the *Wg-Bl-L* chromosome were detected by sequencing DNA amplified from *E-2-iso/CyO-iso* and *Wg-Bl-L-iso/CyO-iso* heterozygous flies (where *iso* indicates an isogenized chromosome): as the *CyO-iso* chromosome is identical in these stocks, any SNPs detected result from differences between the *E-2-iso* and *Wg-Bl-L-iso* chromosomes. SNPs between the *pix^{3c3}* chromosome and the *l(3)s2383^{s2383}-l(3)j2B9^{j2B9}* chromosome were detected by sequencing DNA or by SSCP analysis of DNA amplified from *pix^{3c3}-iso/+iso* and *l(3)s2383^{s2383}-l(3)j2B9^{j2B9}-iso/+iso* flies (where the *+iso* third chromosome was derived from an isogenized *y w* stock). Recombinant chromosomes, in *trans* with either *CyO-iso* or *+iso* third chromosome, were genotyped by RFLPs, SSCPs, or direct DNA sequencing.

Bioinformatics: The design of primer pairs and the determination of SNP locations on the physical map were based on the *D. melanogaster* Genome Release 3 and relied upon the BDGP GadFly annotation database. Orthologs of the Ncm and Prp8 proteins were identified by using the *D. melanogaster* sequences to perform BLASTp searches of the nonredundant peptide databases (http://www.ncbi.nlm.nih.gov/BLAST/). The highest-scoring hits for a variety of species are presented in Figure 7. CLUSTALW (http://pbil.ibcp.fr) was used for sequence alignment and calculation of percentage of identity. Pfam 16.0 (http://www.sanger.ac.uk/Software/Pfam) and SMART (http://smart.embl-heidelberg.de/) were used to identify protein motifs.

RESULTS

Characterization of the *MS1096>Dp110^{KD}* small-wing phenotype: Overexpression of a kinase-dead version of the catalytic subunit of Drosophila PI3K (Dp110^{KD}) has a dominant-negative effect on growth (Leevers *et al.* 1996). Expression of Dp110^{KD} in Drosophila wing imaginal discs using the *MS1096-GAL4* line results in small adult wings as a result of decreased cell size and cell number (Leevers *et al.* 1996). This reduction in wing area is dependent on transgene copy number: female flies heterozygous for a recombinant X chromosome containing *MS1096-GAL4* and *UAS-Dp110^{KD}* (referred to as "*MS1096>Dp110^{KD}*") have wings that are ~25% smaller than those of wild type, whereas wings of *MS1096>Dp110^{KD}* homozygous females or hemizygous males are ~50% smaller (compare Figure 1, A and B, to Figure 5, A and B).

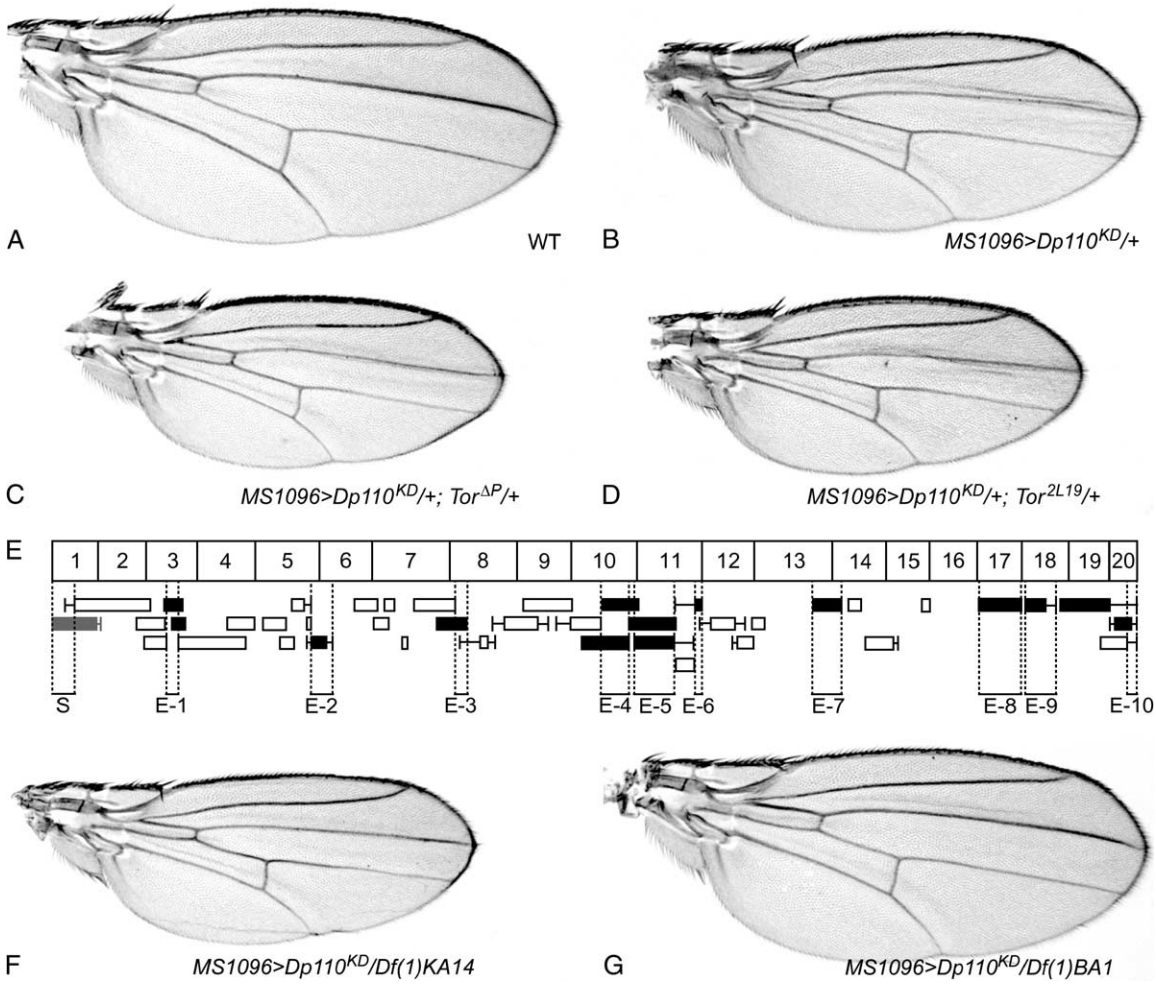

Figure 1.—The small wing induced by overexpression of Dp110^{KD} can be dominantly enhanced or suppressed. (A–D, F, and G) Female wings are shown. (A) Wild type. (B) *MS1096>Dp110^{KD}/+*. (C and D) *Tor* mutations dominantly enhance the small-wing phenotype. (E) Several X chromosome deletions dominantly modify the *MS1096>Dp110^{KD}* small-wing phenotype. Schematic of the X chromosome shows the individual deficiencies tested. Rectangles indicate the regions deleted in each deficiency strain with lines indicating uncertain break points. Vertical dashed lines group together (conservatively) chromosomal regions that, when deleted, either enhance (solid-bar deficiencies; E) or suppress (shaded-bar deficiency; S) the phenotype. The deficiencies corresponding to these regions are listed in materials and methods. (F) An example of a deficiency that dominantly enhances the small-wing phenotype. (G) *Df(1)BA1* dominantly suppresses the small-wing phenotype.

We investigated whether the *MS1096>Dp110^{KD}* small-wing phenotype is sensitive to heterozygous mutation of genes encoding known growth regulators. First, we tested mutations in genes encoding core transducers of InR/PI3K signaling. Surprisingly, null mutations in *Dp110*, *p60*, *Pten*, *Akt1*, and *foxo* each fail to dominantly modify the small-wing phenotype, although *InR^{339}* and *chico^{2}* are weak enhancers (data not shown). We also tested mutations in genes encoding members of the Tor pathway, as this also promotes growth in Drosophila and there are several potential links between Tor and InR/PI3K signaling (Leevers and Hafen 2004). Although strong hypomorphic or null mutations in *Tsc1* or *Rheb* do not dominantly modify *MS1096>Dp110^{KD}* wing size, null mutations in *Tor* clearly enhance the phenotype (Figure 1, C and D). The fact that the majority of InR/PI3K and Tor pathway mutants fail to dominantly modify the small-wing phenotype may be simply because these gene products are not limiting for wing growth, at least under conditions in which InR/PI3K signaling is already impaired. Tor itself may be an exception to this general observation because it promotes growth in response to multiple inputs and has several growth-regulatory targets (Neufeld 2004; see discussion). Together, these observations demonstrate that the small-wing phenotype is relatively insensitive to a reduction in the genetic dose of several well-characterized growth regulators.

We wished to use the *MS1096>Dp110^{KD}* small-wing phenotype in a large-scale modifier screen to identify additional molecules that are important for organ growth *in vivo*. However, in light of the findings described above, we first performed a small-scale pilot test using deficiencies that together span most of the

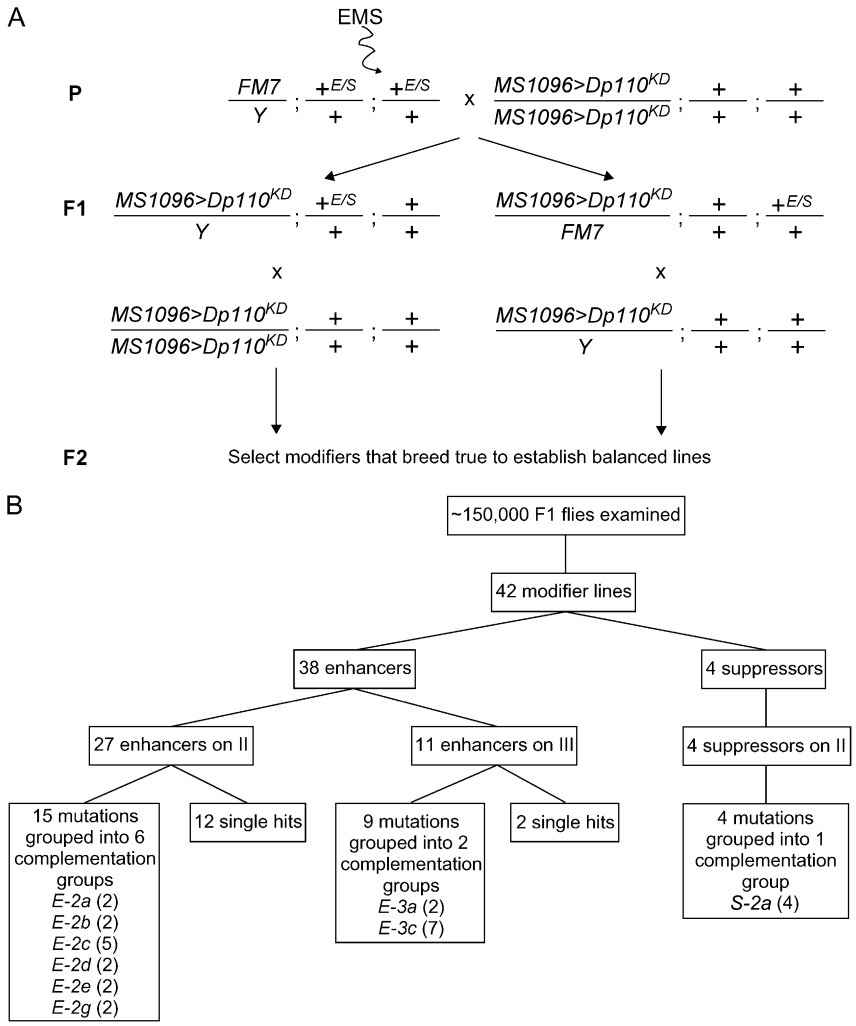

FIGURE 2.—Summary of the screen. (A) Crossing scheme for identifying dominant modifiers of the *MS1096>Dp110^{KD}* small-wing phenotype. EMS-mutagenized *FM7/Y* males were mated with homozygous *MS1096>Dp110^{KD}* virgin females. F_1 flies with mutations that either enhanced or suppressed the small-wing phenotype were individually backcrossed to the *MS1096>Dp110^{KD}* strain to check whether the modifying effect was transmitted to the next generation. The *FM7* chromosome itself does not modify the small-wing phenotype (data not shown). Modifiers that bred true were retained, mapped to chromosomes, and balanced. "*E/S*" represents possible enhancer or suppressor point mutations, respectively, induced by the EMS mutagen. "Y" indicates the Y chromosome and thus identifies the male flies in the crossing scheme. (B) Flow diagram indicating the number and type of modifiers retained at each stage of the initial chromosomal mapping and complementation analyses. Numbers in parentheses in the bottom boxes indicate the number of mutant alleles in each complementation group. Note that the *E-3c* group was subsequently found to comprise two lethal complementation groups of two alleles and three single hits—see text for details.

X chromosome. Fourteen of the 41 deficiencies examined dominantly enhanced the small-wing phenotype whereas one deficiency suppressed it (Figure 1, E–G). Significantly, several of the enhancer deficiencies remove overlapping sections of the chromosome. This means that there are at least 10 regions of the X chromosome that, when deleted, enhance the small-wing phenotype (Figure 1E), suggesting that the *MS1096>Dp110^{KD}* phenotype is indeed sensitive to the dose of other genes. We therefore decided to carry out a larger-scale dominant genetic interaction screen.

Isolation of new growth genes through a dominant modifier screen: Chemically mutagenized males were mated with *MS1096>Dp110^{KD}* homozygous females and the progeny were examined under the dissection microscope for enhancement or suppression of the test phenotype (Figure 2A and MATERIALS AND METHODS). Compared to controls, *MS1096>Dp110^{KD}/Y* male wings are more reduced in size than *MS1096>Dp110^{KD}/+* female wings (see above). We were therefore able to simultaneously screen for modification of our test phenotype at two different strengths (Figure 2A).

The initial results of the screen are summarized in Figure 2B. We screened ~150,000 flies and identified 38 enhancers, collectively termed *E-Dp110^{KD}* mutations, and 4 suppressors, termed *S-Dp110^{KD}* mutations. Most of the modifiers were initially identified in *MS1096>Dp110^{KD}/Y* male wings, suggesting that the stronger male phenotype provided the more effective background in which to detect modifying mutations. Where tested, the majority of these mutations also dominantly modified *MS1096>Dp110^{KD}/+* female wings, although the effect was often weak.

Complementation and preliminary phenotypic analyses: We conducted a lethal complementation analysis among the *E-Dp110^{KD}* and *S-Dp110^{KD}* mutations. As genes required for growth may not be essential for viability (*e.g.*, BOHNI *et al.* 1999; MONTAGNE *et al.* 1999), we also grouped together mutations that, in *trans*, gave rise to viable adults with abnormally sized wings and/or bodies. Mutations that do not display lethality or any obvious size defects when in *trans* with other mutations were classed as "single hits" and were not analyzed further in this study. By these criteria, it is possible that

mutant combinations that cause only mild effects on growth rate or final size may have been missed.

E-Dp110^{KD} mutations on the second chromosome: Complementation analysis of the *E-Dp110^{KD}* mutants mapping to the second chromosome initially yielded six lethal complementation groups named ***E**nhancer on chromosome **2**-complementation groups **a** to **f*** (*E-2a* to *-f*) (Figure 2B). Of these, *E-2c* and *E-2f* were not analyzed further. All five *E-2c* mutations cause ectopic wing vein tissue when combined with *MS1096>Dp110^{KD}* (data not shown), raising the possibility that their ability to reduce *MS1096>Dp110^{KD}* wing size may be an indirect consequence of their effect on patterning. Of the four mutations composing the *E-2f* group, *E-2f^{1}*, *E-2f^{2}*, and *E-2f^{3}* are lethal in *trans* with each other and were found to contain lethal mutations in the *dumpy* gene by complementation analyses (see MATERIALS AND METHODS). However, independently sourced *dumpy* mutations (*dp^{ov1}*, *dp^{lv1}*, and *dp^{olvR}*) fail to enhance the *MS1096>Dp110^{KD}* phenotype (data not shown), suggesting that the *E-2f^{1}*, *E-2f^{2}*, and *E-2f^{3}* chromosomes contain second-site enhancer mutations. Moreover, it was possible to separate the *dumpy* mutation from the *E-Dp110^{KD}* mutation on the *E-2f^{1}* chromosome through recombination (data not shown). Indeed, it has been noted previously that *dumpy* alleles are detected at high rates in EMS mutagenesis screens, probably owing to the enormous size (>100 kb) of the *dumpy* locus (JENKINS 1967; WILKIN *et al.* 2000). Significantly, the *E-2f^{4}* chromosome does not contain a *dumpy* mutation and is lethal in *trans* with *E-2f^{1}* but not with *E-2f^{2}* or *E-2f^{3}*. We surmised that *E-2f^{4}* and the second-site enhancer mutation on the *E-2f^{1}* chromosome are likely to disrupt the same gene and named this distinct enhancer locus "*E-2g*" (Figure 2B). The second-site enhancers on the *E-2f^{2}* and *E-2f^{3}* chromosomes were reclassified as single hits and were not analyzed further.

Phenotypes exhibited by the second chromosome *E-Dp110^{KD}* mutants are consistent with the corresponding wild-type genes encoding proteins with vital or growth-promoting roles. *E-2a trans*-heterozygotes are embryonic lethal, and *E-2b^{1}/E-2b^{2}* animals die either as embryos or soon after hatching (data not shown). In contrast, animals *trans*-heterozygous for *E-2d* or *E-2e* or *E-2g* mutations survive for several days as larvae, although they are abnormally small and never pupate (data not shown). This phenotype is reminiscent of mutations in known growth-promoting genes (*e.g.*, GALLONI and EDGAR 1999; WEINKOVE *et al.* 1999). We also noted that adult flies heterozygous for *E-2b* or *E-2d* mutations have small, slender bristles and show a significant delay in their development to adulthood (MARYGOLD *et al.* 2005). These dominant phenotypes are typical of the *Minute* class of mutations that are thought to disrupt RP genes (LAMBERTSSON 1998) and are therefore consistent with a role for the *E-2b* and *E-2d* products in protein synthesis.

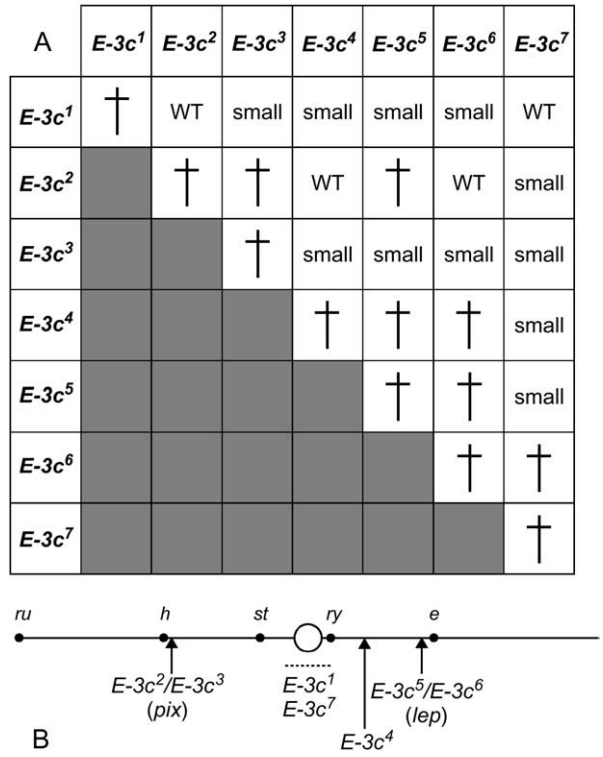

FIGURE 3.—Analysis and mapping of *E-3c* mutations. (A) *E-3c* complementation matrix. Crosses indicate lethality; "small" indicates that *trans*-heterozygous adults had abnormally small bodies and/or wings; WT, wild type. (B) The location of *E-3c* genes on a genetic map of the third chromosome is shown with respect to the *ru h st ry* and *e* recessive markers used in the mapping.

E-Dp110^{KD} mutations on the third chromosome: The *E-Dp110^{KD}* mutations that mapped to the third chromosome did not fall into simple lethal complementation groups. Seven mutations, initially grouped together as *E-3c$^{1-7}$*, each exhibit either lethality or reduced body size when placed in *trans* with at least one other *E-3c* mutation (Figures 2B and 3A). However, the overall complementation pattern is rather complex. For example, *E-3c^{5}* is lethal in *trans* with *E-3c^{2}* or *E-3c^{4}*, but *E-3c^{2}/E-3c^{4} trans*-heterozygotes are phenotypically wild type (Figure 3A). This may indicate either that *E-3c* is a genetically complex single locus or that the *E-3c* group actually comprises several distinct loci that can show particularly strong genetic interactions.

To further investigate the potential allelism of the seven *E-3c* mutations, their ability to enhance the *MS1096>Dp110^{KD}* small-wing phenotype was roughly mapped by meiotic recombination using visible markers (MATERIALS AND METHODS; Figure 3B). *E-3c^{2}* and *E-3c^{3}* each map 1 cM to the right of *hairy* and are lethal in *trans*, indicating that they are likely to disrupt the same gene. Similarly, *E-3c^{5}* and *E-3c^{6}* are probably allelic as each maps ~2 cM to the left of *ebony* and they are lethal in *trans*. We have named the *E-3c^{2}/E-3c^{3}* gene *pixie* (*pix*) and the *E-3c^{5}/E-3c^{6}* gene *leprechaun* (*lep*), owing to the

small body/wing phenotypes produced by these mutations when in *trans* with other *E-3c* mutations or when heterozygous in the *MS1096>Dp110^{KD}* background. Both *E-3c^{1}* and *E-3c^{7}* map close to the centromere but are unlikely to disrupt the same gene as *E-3c^{1}/E-3c^{7}* flies are viable and phenotypically wild type (Figure 3A). Finally, *E-3c^{4}* maps 6 cM to the right of *rosy* and is a single hit. Thus the *E-3c* group appears to comprise mutations at five different loci: *pix*, *lep*, and the three single-hit loci *E-3c^{1}*, *E-3c^{4}*, and *E-3c^{7}* (Figure 3B). We decided to include all the *E-3c* mutations in subsequent analyses, owing to the uniquely strong genetic interactions between them.

Of the lethal combinations among the *E-3c* group, several are larval lethal but survive as abnormally small larvae for an extended period, akin to *E-2d*, *E-2e*, and *E-2g trans*-heterozygotes. Furthermore, both *pix* mutants and *E-3c^{7}* mutants display the dominant Minute phenotypes of small bristles and developmental delay similar to *E-2b* and *E-2d* heterozygotes (data not shown). Together, these observations are consistent with the respective protein products having a positive role in growth.

Four additional *E-Dp110^{KD}* mutations on the third chromosome do not show obvious phenotypes in *trans* with the *E-3c* group of mutations. Among these four, two were provisionally grouped as *E-3a* because they have a *trans*-heterozygous phenotype of small wings and halteres (Figure 2B; data not shown), suggesting that they could affect thoracic disc growth by disrupting the same gene. However, one of these mutant stocks was lost and so *E-3a* could not be analyzed further. The remaining two third chromosome mutants show no phenotype in *trans* with any other third chromosome mutants and were not further analyzed (Figure 2B).

S-Dp110^{KD} mutations: All four suppressors of the *MS1096>Dp110^{KD}* small-wing phenotype are on the second chromosome and form a single semilethal complementation group named *S-2a* (Figure 2B). Various mapping and phenotypic data suggest that *S-2a* corresponds to *apterous* (MATERIALS AND METHODS). Apterous is a transcription factor that directs wing development from the earliest stages and, among other targets, induces expression of *beadex* (MILAN *et al.* 1998). Significantly, the *MS1096-GAL4* transgene used to drive Dp110^{KD} expression in the screen is inserted in the second intron of the *beadex* gene (MILAN *et al.* 1998). We therefore considered it likely that that the *S-2a/apterous* mutations were isolated as suppressors of the *MS1096>Dp110^{KD}* phenotype because they dominantly reduce the expression levels of the *MS1096-GAL4* driver. We therefore did not analyze this group further.

In summary, our screen initially yielded seven complementation groups on the second chromosome (*E-2a*, *E-2b*, *E-2c*, *E-2d*, *E-2e*, *E-2g*, and *S-2a*) and two groups of mutations on the third chromosome (*E-3a* and *E-3c*). Of these, we selected the *E-2a*, *E-2b*, *E-2d*, *E-2e*, *E-2g*, and *E-3c* groups for further analysis (Table 1, column 1). All these mutations were isolated as dominant enhancers of *MS1096>Dp110^{KD}* wing size (Table 1, column 3) and are therefore predicted to disrupt genes encoding proteins that normally promote/permit growth. Other phenotypes presented by these mutants are consistent with this idea (see above).

Dominant effects of the *E-Dp110^{KD}* mutations on wild-type wing size: Before analyzing the *E-Dp110^{KD}* mutations further, we wished to test whether they cause a dominant reduction in wing size in a wild-type genetic background. If this were the case then their apparent ability to dominantly reduce *MS1096>Dp110^{KD}* wing size would be purely an additive effect rather than an informative genetic interaction. We outcrossed the *E-2a*, *E-2b*, *E-2d*, *E-2e*, *E-2g*, and *E-3c* mutations to a wild-type strain and measured wing size in the progeny. None of the *E-Dp110^{KD}* mutations were found to dominantly reduce wing size under standard culture conditions (Table 1, column 5), and they were therefore considered to be *bona fide* enhancers of the *MS1096>Dp110^{KD}* phenotype. However, we did observe that *pix^{3c2}* heterozygotes are reduced in body and wing size when reared under uncrowded, carefully synchronized culture conditions (C. M. A. COELHO, unpublished results). Thus, it is possible that the dominant enhancement of the *MS1096>Dp110^{KD}* small-wing phenotype by the *pix^{3c2}* allele, although not *pix^{3c3}*, is partly the result of an additive effect rather than a genuine genetic interaction. We also noted that several *E-Dp110^{KD}* mutants actually have larger wings than controls when crossed into a wild-type background (data not shown). Regardless of the explanation for this unexpected observation (see DISCUSSION), it makes the ability of these *E-Dp110^{KD}* mutations to dominantly reduce *MS1096>Dp110^{KD}* wing size more emphatic.

Dominant effects of the *E-Dp110^{KD}* mutations on the small-wing phenotype induced by Argos expression: We next examined whether the selected *E-Dp110^{KD}* mutations specifically impair signaling through the InR/PI3K pathway. The Drosophila epidermal growth factor receptor (EGFR) pathway has been shown to promote wing growth (DIAZ-BENJUMEA and HAFEN 1994) and *MS1096-GAL4*-mediated expression of the EGFR inhibitor, Argos (Aos), reduces wing size in addition to affecting patterning of the wing (HOWES *et al.* 1998; Figure 4B). We therefore tested whether the selected *E-Dp110^{KD}* mutations dominantly modify *MS1096>Aos* wing size. Significantly, all *E-Dp110^{KD}* mutations are dominant enhancers of the *MS1096>Aos* small-wing phenotype (Figure 4 and Table 1, column 6). This strongly suggests that the *E-Dp110^{KD}* mutations isolated in our original screen do not specifically affect either InR/PI3K- or EGFR-mediated wing growth. Instead, the *E-Dp110^{KD}* genes are likely to encode factors that either are common to both pathways or perhaps act in parallel growth-promoting pathways. One possible exception to this conclusion is *lep*: *lep^{3c5}* and *lep^{3c6}* are very strong enhancers of *MS1096>Dp110^{KD}* wing size but are among

TABLE 1

***E-Dp110^{KD}* complementation groups and summary of phenotypic analyses**

Complementation group	Alleles	Enhancement of *MS1096>Dp110^{KD}* small wing[a]	Minute bristle phenotype?[b]	Dominant reduction in wing size?	Enhancement of *MS1096>Aos*[c]	
					Small wing?	Wing-vein loss?
E-2a	*E-2a^{1}*	+ + +	No	No	Yes	No
	E-2a^{2}	+ + +	No	No	Yes	No
E-2b	*E-2b^{1}*	+	Yes	No	Yes	No
	E-2b^{2}	+	Yes	No	Yes	No
E-2d	*E-2d^{1}*	+ +	Yes	No	Yes	No
	E-2d^{2}	+ + +	Yes	No	Yes	No
E-2e	*E-2e^{1}*	+	No	No	Yes	No
	E-2e^{2}	+ +	No	No	Yes	No
E-2g	*E-2g^{1}*	+ + +	No	No	Yes	Yes
	E-2g^{2}	+ +	No	No	Yes	Yes
E-3c	*E-3c^{1}*	+ +	No	No	Yes	Yes
	pix^{3c2}	+ + +	Yes	No[d]	Yes	No
	pix^{3c3}	+ +	Yes	No	Yes	No
	E-3c^{4}	+ +	No	No	Yes	No
	lep^{3c5}	+ + +	No	No	Yes	No
	lep^{3c6}	+ + + +	No	No	Yes	No
	E-3c^{7}	+ +	Yes	No	Yes	No

[a] The relative degree of enhancement is a cumulative score that reflects results seen in both female and male wings collected from at least three separate crosses. Approximately, the number of "+" signs corresponds to the mean size of *MS1096>Dp110^{KD}/+*; *E-Dp110^{KD}/+* female wings compared to *MS1096>Dp110^{KD}/+* female wings as follows: +, >95%; ++, 91–95%; +++, 86–90%; ++++, <86%. *P*-values calculated using Student's *t*-test were <0.01 in every case.

[b] Nota were assessed for the Minute small and slender bristle phenotype.

[c] *MS1096>Aos/+*; *E-Dp110^{KD}/+* female wings were compared to *MS1096>Aos/+* female wings. Wings were scored for significant size (mean wing area) and venation (presence and integrity of vein L3) differences.

[d] *pix^{3c2}* heterozygotes do show a mild reduction in wing size when reared under uncrowded, carefully synchronized culture conditions, but not under our standard culture conditions.

the weaker enhancers of *MS1096>Aos* wing size (Figure 5, A–D, and Table 1, column 3; data not shown). This suggests that the *lep* gene product could specifically affect InR/PI3K signaling.

To test this idea, we induced *lep^{3c5}* and *lep^{3c6}* clones in the eye imaginal disc and examined adult eyes for cell size defects. Previous studies have found that adult eye clones that are mutant in *Dp110* or other components of the InR/PI3K pathway show a characteristic reduction in both cell size and clonal growth (*e.g.*, Bohni *et al.* 1999; Weinkove *et al.* 1999). *lep^{3c5}* clones in adult eyes are recovered at a reduced frequency and size compared to their twin spots, consistent with a role for the wild-type *lep* gene product in promoting growth (Figure 5E). However,

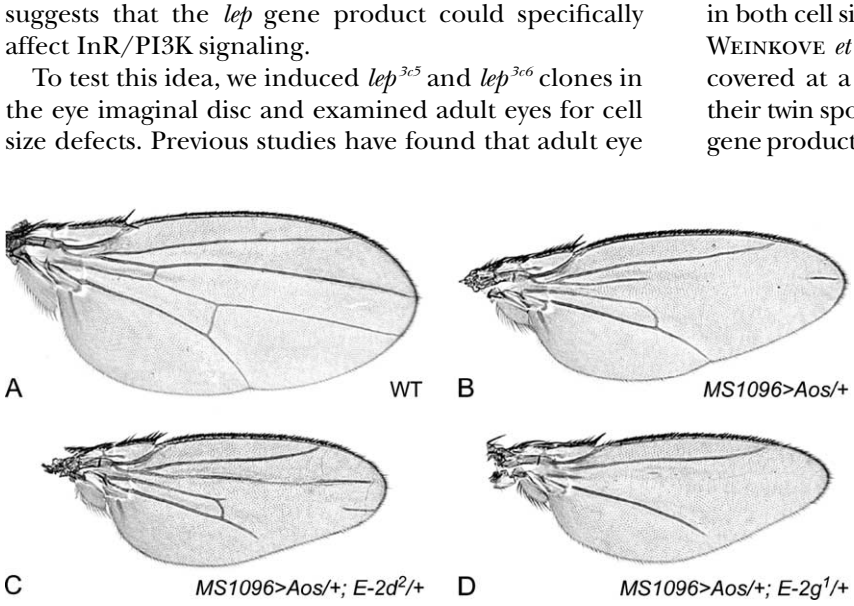

Figure 4.—Dominant modification of *MS1096>Aos* wing phenotypes by *E-Dp110^{KD}* mutations. (A–D) Female wings are shown. (A) Wild type. (B) *MS1096>Aos/+*. (C) Heterozygosity for most *E-Dp110^{KD}* mutations (including *E-2d^{2}*, shown) enhances the size phenotype but does not enhance the venation phenotype. In fact, a slight suppression of the venation phenotype is often observed. (D) Heterozygosity for *E-2g^{1}* enhances both the size and venation phenotypes of the *MS1096>Aos* wing. Similar results were obtained for *E-2g^{2}* and *E-3c^{1}*.

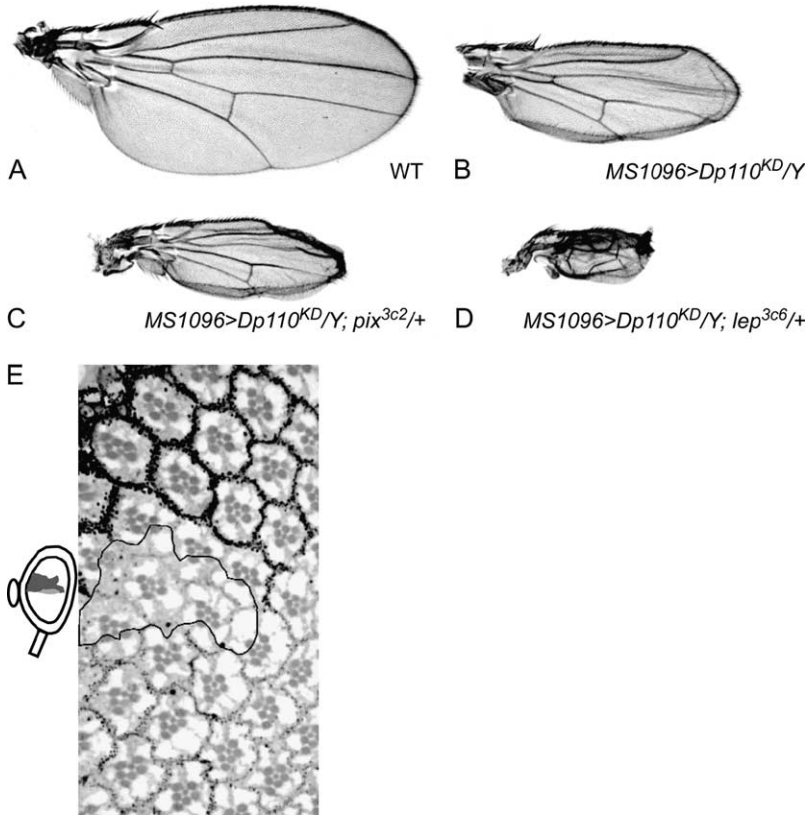

FIGURE 5.—*lep* phenotypes. (A–D) Male wings are shown. (A) Wild type. (B) *MS1096>Dp110^{KD}/Y.* Note that *MS1096>Dp110^{KD}/Y* wings are more reduced in size compared to controls than *MS1096>Dp110^{KD}/+* female wings (compare to Figure 1, A and B), and that the enhancement of the small-wing phenotype is correspondingly stronger in male compared to female wings. (C) *pix^{3c2}* is a strong dominant enhancer of the small-wing phenotype. (D) Heterozygosity for *lep^{3c6}* results in an extreme enhancement of the phenotype and wings are often reduced to stubs (not shown). (E) *lep^{3c5}* clones in the adult eye do not comprise small cells. (Left) Cartoon showing the extent of a $w^{+/+}$ twin spot (dark shading) and its accompanying $w^{-/-}$, *lep^{3c5}* clone (light shading). Most *lep^{3c5}* clones are found at the dorsal-ventral boundary of the eye similar to the one shown. (Right) Cross section of the same eye with the mutant clone marked by absence of pigment granules (outlined). Note that the size and arrangement of the mutant ommatidial cells are similar to those in the surrounding wild-type tissue.

cell size is not detectably reduced in the surviving *lep^{3c5}* clones compared to neighboring wild-type tissue (Figure 5E). *lep^{3c6}* eye clones could not be examined as they do not survive to adulthood. We conclude that Lep is unlikely to act specifically in InR/PI3K signaling but is probably required more generally to promote growth.

Dominant effects of the *E-Dp110^{KD}* mutations on the loss-of-veins phenotype induced by Argos expression: A potential pitfall of screening for modifiers of a phenotype induced by transgene expression is that the isolated mutations affect expression of the transgene rather than modify the test phenotype *per se*. Indeed, we consider that *S-2a/ apterous* was isolated in our screen for this reason (see above). Similarly, the ability of all the selected *E-Dp110^{KD}* mutations to enhance both the *MS1096>Dp110^{KD}* and *MS1096>Aos* small-wing phenotypes may arise simply because they dominantly increase *MS1096-GAL4* expression. If this were so, the *E-Dp110^{KD}* mutations should also enhance other *MS1096-GAL4*-induced phenotypes that are unrelated to growth. We therefore examined whether the distinct loss-of-veins phenotype evident in *MS1096>Aos* wings is enhanced by the selected *E-Dp110^{KD}* mutations (Figure 4; Table 1, column 7). Only the *E-2g* mutations and *E-3c^{1}* significantly enhanced the loss-of-veins phenotype, indicating that these mutations may dominantly increase GAL4 driver expression. Alternatively, these three mutations may somehow affect EGFR-mediated patterning processes in addition to growth. As either explanation is inconsistent with our intention of identifying novel factors that affect growth without disturbing pattern, the *E-3c^{1}* and *E-2g* genes were not analyzed further in this study.

On the basis of the secondary screens described above, we conclude that *E-2a*, *E-2b*, *E-2d*, *E-2e*, *pix*, *lep*, *E-3c^{4}*, and *E-3c^{7}* correspond to *bona fide* growth genes that appear to be generally required to promote growth *in vivo*. We next sought to finely map and thus identify some of these genes, focusing on those represented by more than one mutant allele as this greatly facilitates the mapping process.

***pix* is the ortholog of RLI1:** The ability of both *pix^{3c2}* and *pix^{3c3}* to enhance the small-wing phenotype was mapped initially by meiotic recombination to a region just proximal to *hairy* on chromosome arm 3L (see above and Figure 3B). Complementation tests with deficiencies in the region confirmed this location and delimited the chromosomal region of interest (ROI) containing *pix* to cytological bands 66E3–66F3 (MATERIALS AND METHODS). Higher-resolution mapping of *pix* was achieved using a similar approach to that described in JENNINGS *et al.* (2004). Recombinants were generated between a *pix^{3c3}* chromosome and a chromosome containing two closely spaced *P[w^{+}]* elements that flank the ROI (MATERIALS AND METHODS). In this way, informative recombinants were specifically selected and then genotyped using SNPs (see MATERIALS AND METHODS).

SNP-based mapping of the *pix^{3c3}* mutation showed that the corresponding gene lies in a ~10-kb interval centered at ~3L:8.9 Mb that contains five predicted genes. DNA sequence analysis of these five genes

revealed that *CG5651* contains missense mutations in both *pix* mutant strains. Furthermore, additional mutant alleles of *CG5651*, generated in an independent mutagenesis screen (Dahanukar *et al.* 1999), are lethal in *trans* with pix^{3c2} and pix^{3c3} (C. M. A. Coelho, unpublished results). *CG5651/pix* encodes the Drosophila ortholog of yeast RLI1, a protein implicated in translation initiation and ribosome biogenesis (C. M. A. Coelho, C. Bunn, D. Andersen and S. J. Leevers, unpublished results; Dong *et al.* 2004; Kispal *et al.* 2005; Yarunin *et al.* 2005). A detailed analysis of the *pix* gene and its function will be described elsewhere.

Identification of *E-2a*, *E-2b*, *E-2d*, and *E-2e*: To map the four $E\text{-}Dp110^{KD}$'s on the second chromosome, we adapted the hybrid mapping strategy developed by St. Johnston and colleagues for mapping mutations on the third chromosome (Martin *et al.* 2001). This two-step mapping process initially uses visible markers and a few recombinants to map mutations at a low resolution to a ROI and then uses SNPs and a high density of recombinants within the ROI to map the mutations to high resolution (Figure 6A; materials and methods). In addition, we used small-scale deletion mapping to confirm and sometimes improve upon the SNP-based mapping results.

***E-2e* encodes the splicing factor Prp8:** As illustrated in Figure 6, *E-2e* was mapped by SNP-based methods to a ~94-kb interval centered at ~2R:7.2 Mb (~48E1–4 on the cytological map) that contains 12 predicted genes. This location of *E-2e* was confirmed by deficiency analysis (materials and methods). Two of the 12 candidate genes for *E-2e* were eliminated through lethal complementation tests using preexisting mutations. Of the remaining 10 candidates, only 5 encoded proteins with identifiable Pfam domains, and, of these, 3 were considered to be possible growth genes. Sequencing of these 3 candidate genes in the $E\text{-}2e^{1}$ and $E\text{-}2e^{2}$ mutant chromosomes revealed mutations in the *CG8877* coding sequence (Figure 7A). These are the first reported mutations in this gene.

Conceptual translation of the *CG8877* coding sequence predicts a protein with remarkable similarity to the Prp8 pre-mRNA splicing factor of other species (Mount and Salz 2000; Figure 7A). For example, human and *Saccharomyces cerevisiae* Prp8 are, respectively, 89 and 60% identical to the *D. melanogaster* ortholog over their entire ~2400 amino acids. Indeed, Prp8 proteins are the most evolutionary conserved proteins in the eukaryotic spliceosome and, moreover, Prp8 is one of the most highly conserved nuclear proteins known (Hodges *et al.* 1995; Grainger and Beggs 2005). We have therefore renamed the *D. melanogaster CG8877* gene "*prp8*."

Studies of the yeast and human orthologs have shown that Prp8 has fundamental roles at several steps in spliceosomal assembly and function (reviewed in Grainger and Beggs 2005). It is part of the U5 small nuclear ribonucleoprotein particle (snRNP); the U5·U4/U6 tri-snRNP; and the presplicing, activated, and postsplicing spliceosomal complexes. Furthermore, Prp8 is unique among spliceosomal proteins in that it contacts the intronic branchpoint region and both the 5′ and 3′ splice sites of the pre-mRNA. Thus, Prp8 is thought to provide a vital scaffold and catalytic function in the spliceosome of all eukaryotes. However, despite its remarkable evolutionary conservation, the only obvious motif within Prp8 is a carboxy-terminal MPN/Mov34 domain (Aravind and Ponting 1998). The function of this domain is unclear, but it is also found in proteasome regulatory subunits, eIF-3 subunits,

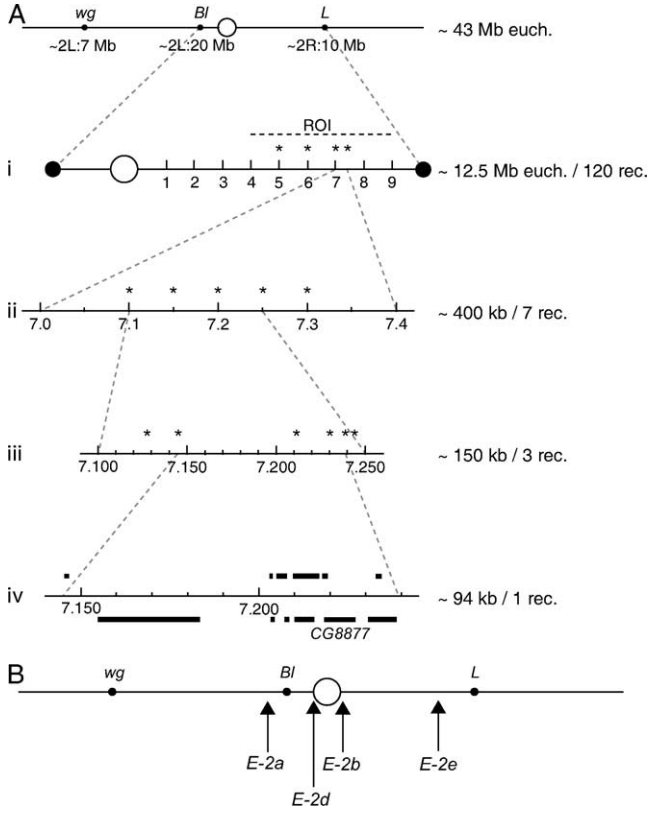

Figure 6.—Mapping $E\text{-}Dp110^{KD}$ mutations on the second chromosome. (A) Summary of the mapping of *E-2e*. A cartoon of the entire second chromosome and successive zooms of particular regions containing *E-2e* are shown on the left. The numbers to the right indicate the size of the euchromatic (euch.) interval known to contain *E-2e* and the number of informative recombinants (rec.) remaining at each stage of the mapping. (i) Initial mapping using dominant visible markers showed *E-2e* to be between *Bristle* and *Lobe*, with greater linkage to the *Lobe* locus at ~2R:10 Mb. SNPs (asterisks) were therefore sought and used for genotyping of recombinants in an ROI of ~2R:4–9 Mb. (ii–iv) Successive rounds of higher-resolution SNP-based mapping. (iv) The ~94-kb region defined by SNP-based mapping contains 12 predicted genes (solid rectangles). Mutations were identified in the *CG8877* coding region in the $E\text{-}2e^{1}$ and $E\text{-}2e^{2}$ lines. Genomic coordinates given in i–iv refer to chromosome arm 2R, are given in megabases, and are derived from Release 3.2 of the genome sequence. (B) The location of $E\text{-}Dp110^{KD}$ genes on a physical map of the second chromosome is shown with respect to the wg^{Sp1}, Bl^{1}, and L^{rm} dominant markers used in the mapping.

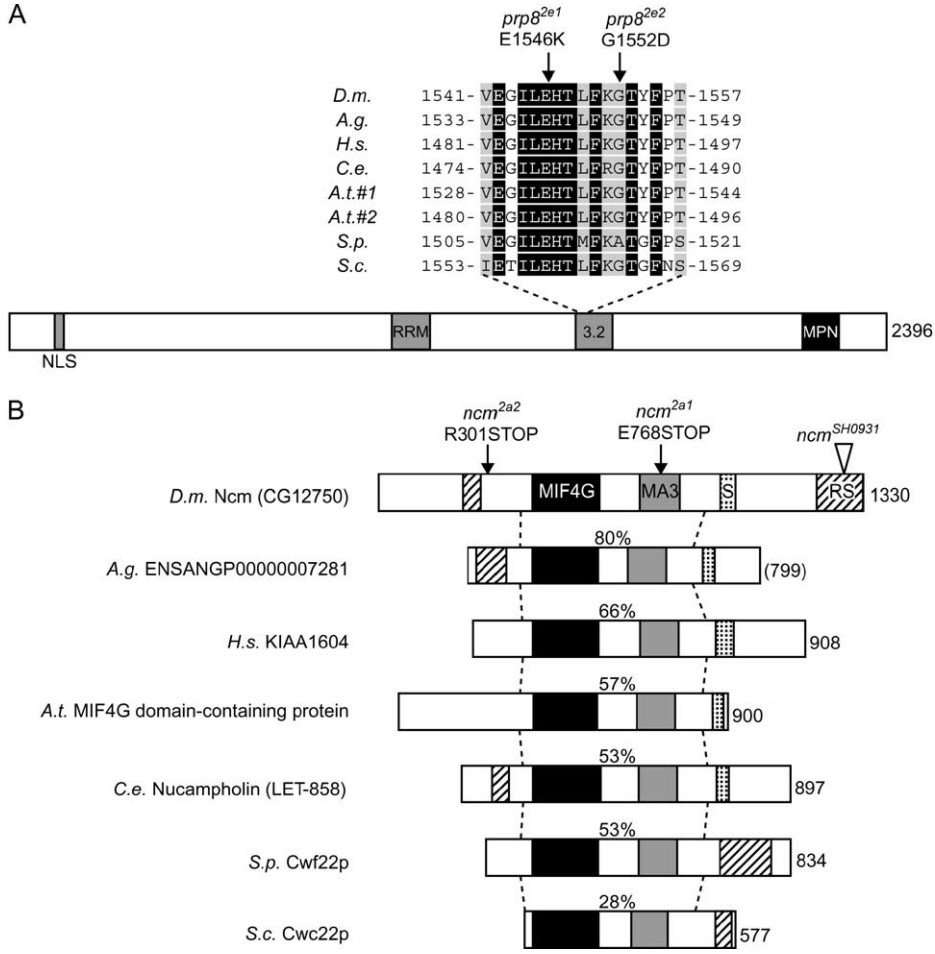

FIGURE 7.—*E-2e* and *E-2a* correspond to the splicing factors Prp8 and Ncm, respectively. (A) Schematic of the *D. melanogaster* Prp8 protein. The $prp8^{2e1}$ and $prp8^{2e2}$ mutations alter highly conserved amino acid residues within the 3′ splice site fidelity region 3.2 (3.2). Also shown is a putative nuclear localization sequence (NLS), a putative RNA recognition motif (RRM), and an MPN domain—see text for details. Amino acid numbering is given for each Prp8 ortholog. Note that the *A. thaliana* genome contains two different genes that encode slightly different Prp8 proteins. (B) Schematic of the *D. melanogaster* Ncm protein and orthologs from different species. Domains shown are: MIF4G domain (solid boxes), MA3 domain (shaded boxes), RS domains (diagonal hatching), and poly-serine tract (stippled). The ncm^{2a2} and ncm^{2a1} mutations each create premature STOP codons at amino acid positions 301 and 768, respectively. The ncm^{SH0931} *P*-element insertion (triangle) is located within the predicted C-terminal RS domain. Ncm orthologs from different species are shown with their percentage identity over a ~500-amino-acid stretch between conserved YIPP and IGLG tetrapeptides in the central region of the *D. melanogaster* protein; amino acid sequences outside of this central sequence are far less conserved. Note that the *Anopheles gambiae* Ncm protein is predicted from a partial cDNA clone that is missing amino- and/or carboxy-terminal sequences. Abbreviations and accession numbers (Prp8/Ncm) are: *D.m.*, *D. melanogaster* (NP_610735.1/NP_609877.2); *A.g.*, *A. gambiae* (XP_308873.1/XP_317618.1); *H.s.*, *Homo sapiens* (NP_006436.2/XP_034594.2); *C.e.*, *C. elegans* (NP_498785.1/NP_496363.1); *A.t.*, *Arabidopsis thaliana* (NP_178124.1 and NP_195589.2/NP_178208.1); *S.p.*, *S. pombe* (NP_593861.1/Q9P6R9); and *S.c.*, *S. cerevisiae* (NP_012035.1/NP_011794.1).

and regulators of transcription factors (ARAVIND and PONTING 1998). Additionally, Grainger and Beggs have recently defined a putative nuclear localization sequence, an RNA recognition motif (RRM), and a third domain they call the "3′ splice site fidelity region" on the basis of the clustering of *S. cerevisiae PRP8* mutations that suppress defects in splicing pre-mRNA 3′ splice site mutations (GRAINGER and BEGGS 2005; Figure 7A). The carboxy-terminal half of this latter region, called "3.2," shows exceptionally high sequence conservation through evolution and has been postulated to act at the catalytic center of the spliceosome (GRAINGER and BEGGS 2005). Remarkably, both the $prp8^{2e1}$ and $prp8^{2e2}$ mutations we identified in *D. melanogaster prp8* result in amino acid substitutions at closely spaced residues within region 3.2 (Figure 7A). Both these mutations are therefore predicted to cripple Prp8 and spliceosomal function and thus cause severe impairment to the expression of all intron-containing pre-mRNAs.

***E-2a* also encodes a splicing factor:** SNP-based mapping of *E-2a* resolved the ROI to a 300-kb interval centered at ~2L:18.4 Mb (~36E–F) containing 17 predicted genes (Figure 6B; data not shown). Deficiency-based mapping further delimited the ROI to an interval of 150 kb containing 9 predicted genes (MATERIALS AND METHODS). DNA sequence analysis of the $E\text{-}2a^1$ and $E\text{-}2a^2$ chromosomes revealed mutations in the *CG12750* coding sequence (Figure 7B). Furthermore, *l(2)SH0931*, a previously reported homozygous lethal *P*-element insertion within *CG12750* (OH *et al.* 2003; Figure 7B), fails to complement both $E\text{-}2a^1$ and $E\text{-}2a^2$ alleles and dominantly reduces $MS1096{>}Dp110^{KD}$ wing size (data not shown). *l(2)SH0931* is a weaker mutation than either $E\text{-}2a^1$ or $E\text{-}2a^2$ because *l(2)SH0931* hemizygotes hatch as slow, sluggish first instars, whereas $E\text{-}2a^1$ or $E\text{-}2a^2$ hemizygotes are embryonic lethal (data not shown).

The CG12750 protein comprises 1330 amino acids and contains a MIF4G domain followed by a MA3 domain in the center of the protein (Figure 7B). MIF4G and MA3 are α-helical domains that are also arranged in tandem in the translation initiation factor eIF-4G

(Ponting 2000). In eIF-4G, the MIF4G domain has been shown to act as a multisubstrate adaptor, binding RNA, DNA, eIF-4A, and eIF-3 (Ponting 2000). Despite the superficial resemblance between eIF-4G and CG12750, BLAST searches show that CG12750 is most similar to a distinct class of proteins that is found across species and includes *Caenorhabditis elegans* Nucampholin/LET-858 (Kelly *et al.* 1997), *Schizosaccharomyces pombe* Cwf22p and *S. cerevisiae* Cwc22p (Ohi *et al.* 2002), and human KIAA1604 protein (Figure 7B). As the *C. elegans* protein was the first member of this protein family to be named, we have also called the *D. melanogaster* ortholog "Nucampholin" (Ncm). The ncm^{2a1} and ncm^{2a2} mutations result in premature truncations of the protein within the MA3 domain and before the MIF4G domain, respectively (Figure 7B).

Several facts indicate that, like Prp8, Ncm and its orthologs perform a key role in pre-mRNA splicing. First, the yeast Cwf22p and Cwc22p proteins were originally identified as components of a Cdc5p/Cef1p-containing splicing complex (Ohi *et al.* 2002) that has since been implicated in the structural rearrangement and activation of the spliceosome (Makarov *et al.* 2002; Chan *et al.* 2003). Second, the KIAA1604 protein was identified in two independent analyses of human spliceosomal components (Jurica *et al.* 2002; Zhou *et al.* 2002). Notably, Moore and colleagues specifically isolated proteins within the spliceosomal C complex, thus placing KIAA1604 in the catalytically competent spliceosome (Jurica *et al.* 2002). Third, *D. melanogaster* Ncm and several Ncm orthologs contain domains rich in alternating arginine and serine residues (RS domains) and/or a poly-serine tract, in addition to the RRM within the MIF4G domain (Pestova *et al.* 1996; Boucher *et al.* 2001; B. J. Blencowe, personal communication) (Figure 7B). Together, these features make Ncm a member of the SR-related protein family of pre-mRNA splicing factors that promote formation of the spliceosomal complex in constitutively spliced pre-mRNAs, as well as regulate splice site selection in alternatively spliced pre-mRNAs (Blencowe *et al.* 1999). Finally, the strong evolutionary conservation between the different Ncm proteins (Figure 7B) further suggests that they play a critical role in splicing. Consistent with this view, *D. melanogaster* ncm^{2a1} and ncm^{2a2} are embryonic lethal mutations and ncm^{2a1} or ncm^{2a2} mutant clones induced in the imaginal wing disc do not survive (data not shown).

***E-2b* and *E-2d* encode RPs:** Both *E-2b* and *E-2d* were mapped to the centric heterochromatin of chromosome 2 (Figure 6B). This chromosomal location limited the resolution of the SNP-based mapping approach as both recombination and SNPs are relatively infrequent near the centromere (Berger *et al.* 2001; Hoskins *et al.* 2001; Martin *et al.* 2001). For this reason, we relied on deficiency-based mapping to improve the mapping resolution of the *E-2b* and *E-2d* genes. Gene identification was further aided by the fact that *E-2b* and *E-2d* mutations cause the dominant Minute bristle phenotype (Table 1, column 4), suggesting that they might disrupt RP-encoding genes (Lambertsson 1998). Indeed, further analyses demonstrated that *E-2b* corresponds to *RpL38* at 41C–E/h46 on 2R, and *E-2d* corresponds to *RpL5* at 40A–B/h35 on 2L. Both these genes encode proteins of the large ribosomal subunit and are thus required for protein synthesis. A detailed analysis of these genes is described elsewhere (Marygold *et al.* 2005).

Mutations in several other RP genes also reduce *MS1096>Dp110^{KD}* wing size: Of the five *E-Dp110^{KD}* genes identified, three encode factors with direct links to mRNA translation (RpL5, RpL38, and Pix), while Prp8 and Ncm are likely to be required in the pre-mRNA splicing process that is prerequisite to efficient translation of most mRNAs (see discussion). We therefore addressed whether other translation factors, and especially RPs, are also limiting for growth of the *MS1096>Dp110^{KD}* wing.

As a first approach, we specifically tested a number of existing mutations in genes encoding translation initiation factors and RPs for their ability to enhance the *MS1096>Dp110^{KD}* small-wing phenotype. Our original screen was not saturating, and so mutations in such genes could have been missed. Hypomorphic mutations in *eIF-3p40*, *eIF-4a*, and *eIF-4E* each fail to dominantly modify the *MS1096>Dp110^{KD}* small-wing phenotype (materials and methods and data not shown). However, this negative result may simply be because these particular mutations do not significantly reduce eIF expression levels (see discussion). In contrast, all 13 RP mutations tested dominantly reduced *MS1096>Dp110^{KD}* wing size (Table 2).

All of the RP mutants tested exhibit the classic Minute phenotypes of developmental delay and/or short, slender bristles, albeit to differing extents (Table 2). Notably, there is a good correlation between the strength of the Minute phenotype and the degree to which the RP mutants dominantly enhance the small-wing phenotype (Table 2). As the strength of Minute phenotype reflects the extent to which protein synthesis is impaired in these animals (*e.g.*, Saeboe-Larssen *et al.* 1998), it follows that the fly's protein synthetic capacity is a major determinant of *MS1096>Dp110^{KD}* wing size. *RpS13^{1}* and *oho23B^{03575}* appear to be exceptions to this general trend (Table 2), perhaps indicating a degree of specificity in the sensitivity of the *MS1096>Dp110^{KD}* wing size to particular RPs. However, these two discrepancies may be explained by the fact that dominant phenotypes, in this case both the Minute phenotype and the degree of enhancement of the small wing, are often subject to the effects of secondary mutations in the genetic background.

As a second way of testing whether lowering the genetic dose of other RPs or translation factors enhances

TABLE 2

RP mutations tested for dominant modification of *MS1096>Dp110^{KD}* wing size

RP	Allele tested	Strength of Minute phenotype[a]	Enhancement of *MS1096>Dp110^{KD}* small wing[b]
RpS3	*RpS3^{1}*	Strong	++++
RpS3A	*RpS3A^{57g}*	Strong	++++
RpS5a	*RpS5a^{1}*	Strong	++++
RpS13	*RpS13^{1}*	Strong	+
RpS17	*RpS17^{6}*	Strong	++++
RpS21	*oho23B^{03575}*	Weak	++
RpS26	*RpS26^{04553}*	Medium	+
	RpS26^{KG00230}	Medium	++
RpLP1	*RpLP1^{beo}*	Medium	++
RpL5[c]	*RpL5^{2d1}*	Medium	++
	RpL5^{2d2}	Medium	+++
RpL38[c]	*RpL38^{2b1}*	Medium	+
	RpL38^{2b2}	Medium	+

[a] Scored as described in MATERIALS AND METHODS.
[b] Scored as described in Table 1, footnote *a*. *P*-values calculated using Student's *t*-test were <0.01 in every case.
[c] Data on *RpL5* and *RpL38* from Table 1 are reproduced here for completeness.

the *MS1096>Dp110^{KD}* small-wing phenotype, we examined whether some of the single hits isolated in the original screen exhibit Minute bristles. Of 10 single-hit mutants examined, 4 have a clear Minute bristle phenotype (data not shown). We conclude that protein synthesis in general and RP levels in particular are limiting for growth under conditions where PI3K-signaling has been compromised.

DISCUSSION

Screening for dominant modifiers of the *MS1096>Dp110^{KD}* small-wing phenotype: We have conducted a screen for genes that are important for organ growth *in vivo*. We used the GAL4/UAS system to overexpress a kinase-dead version of the catalytic subunit of PI3K in the Drosophila wing. The resulting small-wing phenotype provided a sensitized background in which mutations that affect wing growth could be detected. A total of 38 enhancer mutations and 4 suppressor mutations were initially isolated. Various secondary screens were employed that served to focus our studies on those modifiers that were considered most likely to correspond to *bona fide* growth genes. For example, three groups of modifiers (*S-2a*, *E-2g*, and *E-3c^{1}*) were eliminated from further investigation because they were judged likely to affect expression of the *MS1096-GAL4* driver rather than modifying the *MS1096>Dp110^{KD}* wing size phenotype *per se*. Importantly, none of the selected enhancers, with the possible exception of *pix^{3c2}*, dominantly reduced adult wing size in a wild-type background. This demonstrates that these *E-Dp110^{KD}* mutations each reduce *MS1096>Dp110^{KD}* wing size as a result of a genuine genetic interaction rather than as an additive effect, and that their wild-type gene products are limiting for wing growth under conditions in which InR/PI3K signaling is compromised.

TABLE 3

Molecularly identified *E-Dp110^{KD}* genes

Complementation group	Gene disrupted	Predicted molecular function
E-2a	*ncm* (*CG12750*)	Splicing
E-2b	*RpL38* (*CG18001*)	Translation
E-2d	*RpL5* (*CG17489*)	Translation
E-2e	*prp8* (*CG8877*)	Splicing
E-3c^{2}/E-3c^{3}	*pix* (*CG5651*)	Translation

We identified the genes corresponding to five of the *bona fide E-Dp110^{KD}* enhancer groups: two encode splicing factors and the other three encode factors required for protein translation (Table 3). The mutations generated in these five genes have allowed the first genetic and phenotypic characterization of these loci in Drosophila. In addition to these five genes, several other modifiers remain to be identified. These include at least 11 gene regions uncovered by our pilot screen using X chromosome deficiencies, the *lep* gene, and several single-hit loci. On the basis of our preliminary characterization of the *MS1096>Dp110^{KD}* small-wing phenotype, a mutation in *Tor* or possibly *chico* or *InR* might be found among the single-hit enhancers, although this has not been tested.

Although a large number of flies were screened, relatively few modifying mutations were identified, relatively small complementation groups were obtained, and several mutations that might have been predicted to have a dominant modifying effect did not do so. One trivial explanation for the former two of these observations is that we were screening for a quantitative phenotype (wing area) that lacked an internal control, meaning that subtle interactions could have been missed. It is also possible that only a restricted set of factors—perhaps those that integrate a number of growth regulatory signals—are limiting for growth of the *MS1096>Dp110^{KD}* wing. This idea is consistent with the fact that over half of the mutations isolated in the original screen were put into just 10 complementation groups. Furthermore, of mutations in several core members of the InR/PI3K or Tor pathways, only mutations in *Tor* itself, which promotes growth in response to multiple inputs and has several growth regulatory targets, clearly dominantly affected *MS1096>Dp110^{KD}* wing size.

A third possibility that could explain the output of our screen is that the *MS1096>Dp110^{KD}* small-wing phenotype or growth in general is relatively insensitive to small changes in gene dosage/protein levels. In this scenario, our screen would have been biased toward detecting

rare antimorphic mutations or hypomorphic/null mutations of haplo-insufficient genes. Indeed, our analyses of the five *E-Dp110^{KD}* genes we identified support this hypothesis. For example, genomic deletions that remove the *pix* locus do not dominantly enhance the *MS1096>Dp110^{KD}* small-wing phenotype, and flies heterozygous for the deletion have a weaker growth phenotype than flies heterozygous for the *pix^{3c2}* or *pix^{3c3}* mutations (C. M. A. Coelho, unpublished data). Also, the various RP mutants identified as enhancers in this study are all dominant mutations as a result of the haploinsufficiency of these loci. Finally, both *ncm* mutations result in premature STOP codons within the coding sequence of the gene that may produce truncated Ncm proteins that could act as dominant-negative proteins.

The role of splicing and translation in organ growth: It is well established that cellular and organ growth depends upon the synthesis of new proteins (Jorgensen *et al.* 2004). Indeed, several growth-stimulatory pathways, including InR/PI3K signaling, are known to result in increased ribosome biogenesis and/or protein synthesis (Thomas 2000). From this perspective, it is perhaps not surprising that the *MS1096>Dp110^{KD}* small-wing phenotype is enhanced by mutations in genes encoding factors required for efficient protein synthesis, such as RPs or Pix. Alternatively, it is possible that suboptimal levels/activity of Pix or certain RPs result in lowered synthesis of a more specific set of growth regulatory targets, as has been proposed for eIF-4a (Galloni and Edgar 1999).

Although translation initiation is thought to be the rate-limiting step in cellular protein synthesis, it is at least feasible that disruption of the preceding steps, such as the processing of pre-mRNA to form mature mRNA transcripts, can also reduce the overall rate of protein production (Matthews *et al.* 2000). Thus, the identification of splicing factors in our growth screen may reflect that protein synthesis at the level of transcript maturation can be limiting for organ growth. This might especially be the case for *prp8* mutant heterozygotes. Here, a 50% reduction in functional Prp8 protein would be expected to decrease the splicing efficiency of all intron-containing pre-mRNAs, but would particularly impair the expression of highly expressed genes required for growth and protein synthesis, such as RP transcripts. Indeed, it is known that expression of certain RPs is controlled at the level of splicing (Jorgensen *et al.* 2004). However, we note that neither *ncm* nor *prp8* mutants exhibit a dominant Minute bristle phenotype (Table 1, column 4), suggesting that protein synthesis is not severely compromised in these heterozygous mutant flies.

An alternative explanation for the identification of Prp8 and Ncm in our screen would be that they specifically disrupt splicing of pre-mRNAs that act in growth-promoting pathways: many "growth genes" can be alternatively spliced to produce different protein isoforms and abnormal expression/phosphorylation of splicing factors has been observed in certain cancers (Kalnina *et al.* 2005). Although such a mechanism seems unlikely for a core spliceosomal protein such as Prp8, it is an attractive hypothesis in the case of the RS domain-containing Ncm protein. Interestingly, the activity of RS domain splicing factors can be regulated by phosphorylation (Stamm 2002) and the RS domains themselves are potentially substrates for Akt kinase as they often contain sequences that conform to the Akt optimal phosphorylation motif of RXRXXS/T (Obata *et al.* 2000). Indeed, the amino- and carboxy-terminal RS domains of *D. melanogaster* Ncm are predicted to contain 10 potential phosphorylation sites for Akt under high-stringency conditions (http://scansite.mit.edu/). As Akt is itself activated by InR/PI3K signaling, Ncm could feasibly regulate the production of specific splice variants that promote growth in an InR/PI3K-dependent manner. Although this idea remains to be tested, a precedent exists in the case of PI3K-dependent regulation of the human SR protein SRp40. Here, activation of InR/PI3K signaling results in phosphorylation of SRp40 by Akt; phospho-SRp40 then acts on an intronic element in the PKCβII pre-mRNA to promote incorporation of exon 2 into the mature PKCβII transcript (Patel *et al.* 2005).

Surprisingly, we found that several *E-Dp110^{KD}* mutations, including those in *ncm*, *prp8*, *pix*, *RpL5*, and *RpL38*, can dominantly increase wing size in the absence of the *MS1096>Dp110^{KD}* transgenes (data not shown; Marygold *et al.* 2005), suggesting that these *E-Dp110^{KD}* gene products limit growth in a wild-type background. Although we do not understand the mechanism involved, this unexpected observation may be the end result of suboptimal rates of splicing or translation throughout the development of these flies: this may disturb the normal tight regulation of growth, leading to an increase in final organ size. Indeed, there are a number of precedents for tissue overgrowth occurring despite an overall reduction in the rate/level of protein synthesis. For example, decreased levels of any of several different RPs can give rise to tumors in zebrafish (Amsterdam *et al.* 2004), and mutation of Drosophila *RpS21* (Torok *et al.* 1999) or *RpS6* (Watson *et al.* 1992; Stewart and Denell 1993) results in overgrowth of the imaginal discs and/or hematopoietic organs. Quite how certain *E-Dp110^{KD}* mutations dominantly increase wing size in a wild-type background yet dominantly reduce *MS1096>Dp110^{KD}* wing size is a mystery at the present time. Further insight into this apparent paradox will require study of the growth of *E-Dp110^{KD}* mutants, with and without the *MS1096>Dp110^{KD}* transgenes, at earlier time points in wing development.

Identifying novel growth genes in Drosophila: Genetic screens have successfully identified a number of different growth promoters and growth inhibitors in Drosophila (see Introduction). It is evident from these

studies that screens of a similar type have often yielded the same growth genes. For example, several independent clonal screens in the eye identified *Tsc1* (Gao and Pan 2001; Potter *et al.* 2001; Tapon *et al.* 2001), *salvador* (Kango-Singh *et al.* 2002; Tapon *et al.* 2002), and *hippo* (Harvey *et al.* 2003; Jia *et al.* 2003; Udan *et al.* 2003; Wu *et al.* 2003). Similarly, multiple overexpression screens isolated *bantam* (Hipfner *et al.* 2002; Raisin *et al.* 2003) and *Rheb* (Patel *et al.* 2003; Saucedo *et al.* 2003; Stocker *et al.* 2003). It is equally evident that different types of growth regulator have been discovered through different types of growth screen. Indeed, the screen we describe in this article is different from those conducted previously, and the growth genes we have isolated have not been detected in other screens. One explanation for this is that diverse growth genes are susceptible to discovery only in certain types of screen. That is, an obvious growth phenotype may be obtained through gain-of-function but not loss-of-function experiments, through clonal but not whole-organ analyses, or through studies in the wing but not the eye. For example, it is unlikely that RP gene mutations could be identified in either clonal or overexpression screens because homozygous mutant clones are cell lethal whereas overexpression of a single RP is not expected to be able to promote growth (Lambertsson 1998). Indeed, to our knowledge, splicing factors have not previously been identified to be limiting for growth, while core translation factors have been isolated in only two other Drosophila growth screens: mutations in *eIF-4a*, *bonsai/ mRpS15*, and possibly *RpL30* were identified in a screen for growth-defective homozygous mutant larvae (Galloni and Edgar 1999), while a mutation in *mRpL12* was identified as a suppressor of a CycD–Cdk4 overexpression phenotype (Frei *et al.* 2005). We envisage that more context- and screen-dependent growth genes are to be discovered.

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LITERATURE CITED

Amsterdam, A., K. C. Sadler, K. Lai, S. Farrington, R. T. Bronson *et al.*, 2004 Many ribosomal protein genes are cancer genes in zebrafish. PLoS Biol. **2:** E139.

Aravind, L., and C. P. Ponting, 1998 Homologues of 26S proteasome subunits are regulators of transcription and translation. Protein Sci. **7:** 1250–1254.

Berger, J., T. Suzuki, K. A. Senti, J. Stubbs, G. Schaffner *et al.*, 2001 Genetic mapping with SNP markers in *Drosophila*. Nat. Genet. **29:** 475–481.

Blencowe, B. J., J. A. Bowman, S. McCracken and E. Rosonina, 1999 SR-related proteins and the processing of messenger RNA precursors. Biochem. Cell Biol. **77:** 277–291.

Bohni, R., J. Riesgo-Escovar, S. Oldham, W. Brogiolo, H. Stocker *et al.*, 1999 Autonomous control of cell and organ size by CHICO, a *Drosophila* homolog of vertebrate IRS1–4. Cell **97:** 865–875.

Boucher, L., C. A. Ouzounis, A. J. Enright and B. J. Blencowe, 2001 A genome-wide survey of RS domain proteins. RNA **7:** 1693–1701.

Bradley, G. L., and S. J. Leevers, 2003 Amino acids and the humoral regulation of growth: fat bodies use slimfast. Cell **114:** 656–658.

Brennecke, J., D. R. Hipfner, A. Stark, R. B. Russell and S. M. Cohen, 2003 *bantam* encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene *hid* in *Drosophila*. Cell **113:** 25–36.

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

Bryant, P. J., K. L. Watson, R. W. Justice and D. F. Woods, 1993 Tumor suppressor genes encoding proteins required for cell interactions and signal transduction in *Drosophila*. Dev. Suppl., 239–249.

Capdevila, J., and I. Guerrero, 1994 Targeted expression of the signaling molecule decapentaplegic induces pattern duplications and growth alterations in *Drosophila* wings. EMBO J. **13:** 4459–4468.

Carlson, E. A., 1959 Allelism, complementation, and pseudoallelism at the *dumpy* locus of *Drosophila melanogaster*. Genetics **44:** 347–373.

Chan, S. P., D. I. Kao, W. Y. Tsai and S. C. Cheng, 2003 The Prp19p-associated complex in spliceosome activation. Science **302:** 279–282.

Colombani, J., S. Raisin, S. Pantalacci, T. Radimerski, J. Montagne *et al.*, 2003 A nutrient sensor mechanism controls *Drosophila* growth. Cell **114:** 739–749.

Dahanukar, A., J. A. Walker and R. P. Wharton, 1999 Smaug, a novel RNA-binding protein that operates a translational switch in *Drosophila*. Mol. Cell **4:** 209–218.

Datar, S. A., H. W. Jacobs, A. F. de la Cruz, C. F. Lehner and B. A. Edgar, 2000 The *Drosophila* cyclin D-Cdk4 complex promotes cellular growth. EMBO J. **19:** 4543–4554.

de la Cova, C., M. Abril, P. Bellosta, P. Gallant and L. A. Johnston, 2004 *Drosophila myc* regulates organ size by inducing cell competition. Cell **117:** 107–116.

Diaz-Benjumea, F. J., and E. Hafen, 1994 The *sevenless* signalling cassette mediates *Drosophila* EGF receptor function during epidermal development. Development **120:** 569–578.

Dong, J., R. Lai, K. Nielsen, C. A. Fekete, H. Qiu *et al.*, 2004 The essential ATP-binding cassette protein RLI1 functions in translation by promoting preinitiation complex assembly. J. Biol. Chem. **279:** 42157–42168.

Edgar, B. A., and H. F. Nijhout, 2004 Growth and cell cycle control in *Drosophila*, pp. 23–83 in *Cell Growth*, edited by M. N. Hall, M. Raff and G. Thomas. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Fernandez, R., D. Tabarini, N. Azpiazu, M. Frasch and J. Schlessinger, 1995 The *Drosophila* insulin receptor homolog: a gene essential for embryonic development encodes two receptor isoforms with different signaling potential. EMBO J. **14:** 3373–3384.

Frei, C., and B. A. Edgar, 2004 *Drosophila* cyclin D/Cdk4 requires Hif-1 prolyl hydroxylase to drive cell growth. Dev. Cell **6:** 241–251.

Frei, C., M. Galloni, E. Hafen and B. A. Edgar, 2005 The *Drosophila* mitochondrial ribosomal protein mRpL12 is required for Cyclin D/Cdk4-driven growth. EMBO J. **24:** 623–634.

Galloni, M., and B. A. Edgar, 1999 Cell-autonomous and non-autonomous growth-defective mutants of *Drosophila melanogaster*. Development **126:** 2365–2375.

Gao, X., and D. Pan, 2001 TSC1 and TSC2 tumor suppressors antagonize insulin signaling in cell growth. Genes Dev. **15:** 1383–1392.

Gao, X., T. P. Neufeld and D. Pan, 2000 *Drosophila* PTEN regulates cell growth and proliferation through PI3K-dependent and -independent pathways. Dev. Biol. **221:** 404–418.

Goberdhan, D. C., N. Paricio, E. C. Goodman, M. Mlodzik and C. Wilson, 1999 *Drosophila* tumor suppressor PTEN controls cell size and number by antagonizing the Chico/PI3-kinase signaling pathway. Genes Dev. **13:** 3244–3258.

Grainger, R. J., and J. D. Beggs, 2005 Prp8 protein: at the heart of the spliceosome. RNA **11:** 533–557.

Harvey, K. F., C. M. Pfleger and I. K. Hariharan, 2003 The *Drosophila* Mst ortholog, *hippo*, restricts growth and cell proliferation and promotes apoptosis. Cell **114:** 457–467.

Hipfner, D. R., K. Weigmann and S. M. Cohen, 2002 The *bantam* gene regulates Drosophila growth. Genetics **161:** 1527–1537.

Hodges, P. E., S. P. Jackson, J. D. Brown and J. D. Beggs, 1995 Extraordinary sequence conservation of the PRP8 splicing factor. Yeast **11:** 337–342.

Hoskins, R. A., A. C. Phan, M. Naeemuddin, F. A. Mapa, D. A. Ruddy *et al.*, 2001 Single nucleotide polymorphism markers for genetic mapping in *Drosophila melanogaster*. Genome Res. **11:** 1100–1113.

Howes, R., J. D. Wasserman and M. Freeman, 1998 In vivo analysis of Argos structure-function. Sequence requirements for inhibition of the *Drosophila* epidermal growth factor receptor. J. Biol. Chem. **273:** 4275–4281.

Ito, N., and G. M. Rubin, 1999 *gigas*, a *Drosophila* homolog of tuberous sclerosis gene product-2, regulates the cell cycle. Cell **96:** 529–539.

Jenkins, J. B., 1967 Mutagenesis at a complex locus in *Drosophila* with the monofunctional alkylating agent, ethyl methanesulfonate. Genetics **57:** 783–793.

Jennings, B. H., S. Shah, Y. Yamaguchi, M. Seki, R. G. Phillips *et al.*, 2004 Locus-specific requirements for Spt5 in transcriptional activation and repression in *Drosophila*. Curr. Biol. **14:** 1680–1684.

Jia, J., W. Zhang, B. Wang, R. Trinko and J. Jiang, 2003 The *Drosophila* Ste20 family kinase dMST functions as a tumor suppressor by restricting cell proliferation and promoting apoptosis. Genes Dev. **17:** 2514–2519.

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

Johnston, L. A., D. A. Prober, B. A. Edgar, R. N. Eisenman and P. Gallant, 1999 *Drosophila myc* regulates cellular growth during development. Cell **98:** 779–790.

Jorgensen, P., M. Tyers and J. R. Warner, 2004 Forging the factory: ribosome synthesis and growth control in budding yeast, pp. 329–370 in *Cell Growth*, edited by M. N. Hall, M. Raff and G. Thomas. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Junger, M. A., F. Rintelen, H. Stocker, J. D. Wasserman, M. Vegh *et al.*, 2003 The *Drosophila* forkhead transcription factor FOXO mediates the reduction in cell number associated with reduced insulin signaling. J. Biol. **2:** 20.

Jurica, M. S., L. J. Licklider, S. R. Gygi, N. Grigorieff and M. J. Moore, 2002 Purification and characterization of native spliceosomes suitable for three-dimensional structural analysis. RNA **8:** 426–439.

Justice, R. W., O. Zilian, D. F. Woods, M. Noll and P. J. Bryant, 1995 The *Drosophila* tumor suppressor gene warts encodes a homolog of human myotonic dystrophy kinase and is required for the control of cell shape and proliferation. Genes Dev. **9:** 534–546.

Kalnina, Z., P. Zayakin, K. Silina and A. Line, 2005 Alterations of pre-mRNA splicing in cancer. Genes Chromosomes Cancer **42:** 342–357.

Kango-Singh, M., R. Nolo, C. Tao, P. Verstreken, P. R. Hiesinger *et al.*, 2002 Shar-pei mediates cell proliferation arrest during imaginal disc growth in *Drosophila*. Development **129:** 5719–5730.

Kelly, W. G., S. Xu, M. K. Montgomery and A. Fire, 1997 Distinct requirements for somatic and germline expression of a generally expressed *Caernorhabditis elegans* gene. Genetics **146:** 227–238.

Kispal, G., K. Sipos, H. Lange, Z. Fekete, T. Bedekovics *et al.*, 2005 Biogenesis of cytosolic ribosomes requires the essential iron-sulphur protein Rli1p and mitochondria. EMBO J. **24:** 589–598.

Lambertsson, A., 1998 The *Minute* genes in *Drosophila* and their molecular functions. Adv. Genet. **38:** 69–134.

Leevers, S. J., and E. Hafen, 2004 Growth regulation by insulin and TOR signalling in *Drosophila*, pp.167–192 in *Cell Growth*, edited by M. N. Hall, M. Raff and G. Thomas. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Leevers, S. J., D. Weinkove, L. K. MacDougall, E. Hafen and M. D. Waterfield, 1996 The *Drosophila* phosphoinositide 3-kinase Dp110 promotes cell growth. EMBO J. **15:** 6584–6594.

Lindsley, D. L., and G. G. Zimm, 1992 *The Genome of Drosophila melanogaster*. Academic Press, San Diego.

MacDougall, L. K., M. E. Gagou, S. J. Leevers, E. Hafen and M. D. Waterfield, 2004 Targeted expression of the class II phosphoinositide 3-kinase in *Drosophila melanogaster* reveals lipid kinase-dependent effects on patterning and interactions with receptor signaling pathways. Mol. Cell. Biol. **24:** 796–808.

Makarov, E. M., O. V. Makarova, H. Urlaub, M. Gentzel, C. L. Will *et al.*, 2002 Small nuclear ribonucleoprotein remodeling during catalytic activation of the spliceosome. Science **298:** 2205–2208.

Martin, S. G., K. C. Dobi and D. St. Johnston, 2001 A rapid method to map mutations in *Drosophila*. Genome Biol. **2:** RESEARCH0036.

Marygold, S. J., and S. J. Leevers, 2002 Growth signaling: TSC takes its place. Curr. Biol. **12:** R785–R787.

Marygold, S. J., C. M. Coelho and S. J. Leevers, 2005 Genetic analysis of *RpL38* and *RpL5*, two *Minute* genes located in the centric heterochromatin of chromosome 2 of *Drosophila melanogaster*. Genetics **169:** 683–695.

Matthews, M. B., N. Sonenberg and J. W. B. Hershey, 2000 Origins and principles of translational control, pp. 1–31 in *Translational Control of Gene Expression*, edited by N. Sonenberg, J. W. B. Hershey and M. B. Matthews. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Meyer, C. A., H. W. Jacobs, S. A. Datar, W. Du, B. A. Edgar *et al.*, 2000 *Drosophila* Cdk4 is required for normal growth and is dispensable for cell cycle progression. EMBO J. **19:** 4533–4542.

Milan, M., F. J. Diaz-Benjumea and S. M. Cohen, 1998 *Beadex* encodes an LMO protein that regulates Apterous LIM-homeodomain activity in *Drosophila* wing development: a model for LMO oncogene function. Genes Dev. **12:** 2912–2920.

Montagne, J., M. J. Stewart, H. Stocker, E. Hafen, S. C. Kozma *et al.*, 1999 *Drosophila* S6 kinase: a regulator of cell size. Science **285:** 2126–2129.

Mount, S. M., and H. K. Salz, 2000 Pre-messenger RNA processing factors in the *Drosophila* genome. J. Cell Biol. **150:** F37–F44.

Neufeld, T. P., 2004 Genetic analysis of TOR signaling in *Drosophila*. Curr. Top. Microbiol. Immunol. **279:** 139–152.

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

Newsome, T. P., B. Åsling and B. J. Dickson, 2000 Analysis of *Drosophila* photoreceptor axon guidance in eye-specific mosaics. Development **127:** 851–860.

Obata, T., M. B. Yaffe, G. G. Leparc, E. T. Piro, H. Maegawa *et al.*, 2000 Peptide and protein library screening defines optimal substrate motifs for AKT/PKB. J. Biol. Chem. **275:** 36108–36115.

Oh, S. W., T. Kingsley, H. H. Shin, Z. Zheng, H. W. Chen *et al.*, 2003 A *P*-element insertion screen identified mutations in 455 novel essential genes in Drosophila. Genetics **163:** 195–201.

Ohi, M. D., A. J. Link, L. Ren, J. L. Jennings, W. H. McDonald *et al.*, 2002 Proteomics analysis reveals stable multiprotein complexes in both fission and budding yeasts containing Myb-related Cdc5p/Cef1p, novel pre-mRNA splicing factors, and snRNAs. Mol. Cell. Biol. **22:** 2011–2024.

Oldham, S., J. Montagne, T. Radimerski, G. Thomas and E. Hafen, 2000 Genetic and biochemical characterization of dTOR, the *Drosophila* homolog of the target of rapamycin. Genes Dev. **14:** 2689–2694.

Patel, N. A., S. Kaneko, H. S. Apostolatos, S. S. Bae, J. E. Watson *et al.*, 2005 Molecular and genetic studies imply Akt-mediated signaling promotes protein kinase CbetaII alternative splicing via phosphorylation of serine/arginine-rich splicing factor SRp40. J. Biol. Chem. **280:** 14302–14309.

Patel, P. H., N. Thapar, L. Guo, M. Martinez, J. Maris *et al.*, 2003 *Drosophila* Rheb GTPase is required for cell cycle progression and cell growth. J. Cell Sci. **116:** 3601–3610.

Pestova, T. V., I. N. Shatsky and C. U. Hellen, 1996 Functional dissection of eukaryotic initiation factor 4F: the 4A subunit

and the central domain of the 4G subunit are sufficient to mediate internal entry of 43S preinitiation complexes. Mol. Cell. Biol. **16:** 6870–6878.

Ponting, C. P., 2000 Novel eIF4G domain homologues linking mRNA translation with nonsense-mediated mRNA decay. Trends Biochem. Sci. **25:** 423–426.

Potter, C. J., H. Huang and T. Xu, 2001 *Drosophila* Tsc1 functions with Tsc2 to antagonize insulin signaling in regulating cell growth, cell proliferation, and organ size. Cell **105:** 357–368.

Prober, D. A., and B. A. Edgar, 2000 Ras1 promotes cellular growth in the *Drosophila* wing. Cell **100:** 435–446.

Raisin, S., S. Pantalacci, J. P. Breittmayer and P. Leopold, 2003 A new genetic locus controlling growth and proliferation in *Drosophila melanogaster*. Genetics **164:** 1015–1025.

Reiling, J. H., and E. Hafen, 2004 The hypoxia-induced paralogs Scylla and Charybdis inhibit growth by down-regulating S6K activity upstream of TSC in *Drosophila*. Genes Dev. **18:** 2879–2892.

Ryoo, H. D., and H. Steller, 2003 Hippo and its mission for growth control. Nat. Cell Biol. **5:** 853–855.

Saeboe-Larssen, S., M. Lyamouri, J. Merriam, M. P. Oksvold and A. Lambertsson, 1998 Ribosomal protein insufficiency and the Minute syndrome in Drosophila: a dose-response relationship. Genetics **148:** 1215–1224.

Saucedo, L. J., X. Gao, D. A. Chiarelli, L. Li, D. Pan *et al.*, 2003 Rheb promotes cell growth as a component of the insulin/TOR signalling network. Nat. Cell Biol. **5:** 566–571.

Stamm, S., 2002 Signals and their transduction pathways regulating alternative splicing: a new dimension of the human genome. Hum. Mol. Genet. **11:** 2409–2416.

Staveley, B. E., L. Ruel, J. Jin, V. Stambolic, F. G. Mastronardi *et al.*, 1998 Genetic analysis of protein kinase B (AKT) in *Drosophila*. Curr. Biol. **8:** 599–602.

Stewart, M. J., and R. Denell, 1993 Mutations in the *Drosophila* gene encoding ribosomal protein S6 cause tissue overgrowth. Mol. Cell. Biol. **13:** 2524–2535.

Stocker, H., T. Radimerski, B. Schindelholz, F. Wittwer, P. Belawat *et al.*, 2003 Rheb is an essential regulator of S6K in controlling cell growth in *Drosophila*. Nat. Cell Biol. **5:** 559–565.

Tapon, N., N. Ito, B. J. Dickson, J. E. Treisman and I. K. Hariharan, 2001 The *Drosophila* tuberous sclerosis complex gene homologs restrict cell growth and cell proliferation. Cell **105:** 345–355.

Tapon, N., K. F. Harvey, D. W. Bell, D. C. Wahrer, T. A. Schiripo *et al.*, 2002 *salvador* promotes both cell cycle exit and apoptosis in *Drosophila* and is mutated in human cancer cell lines. Cell **110:** 467–478.

Thomas, G., 2000 An encore for ribosome biogenesis in the control of cell proliferation. Nat. Cell Biol. **2:** E71–E72.

Tomlinson, A., and D. F. Ready, 1987 Neuronal differentiation in the *Drosophila* ommatidium. Dev. Biol. **120:** 366–376.

Torok, I., D. Herrmann-Horle, I. Kiss, G. Tick, G. Speer *et al.*, 1999 Down-regulation of RpS21, a putative translation initiation factor interacting with P40, produces viable Minute imagos and larval lethality with overgrown hematopoietic organs and imaginal discs. Mol. Cell. Biol. **19:** 2308–2321.

Udan, R. S., M. Kango-Singh, R. Nolo, C. Tao and G. Halder, 2003 Hippo promotes proliferation arrest and apoptosis in the Salvador/Warts pathway. Nat. Cell Biol. **5:** 914–920.

Watson, K. L., K. D. Konrad, D. F. Woods and P. J. Bryant, 1992 *Drosophila* homolog of the human S6 ribosomal protein is required for tumor suppression in the hematopoietic system. Proc. Natl. Acad. Sci. USA **89:** 11302–11306.

Watson, K. L., R. W. Justice and P. J. Bryant, 1994 *Drosophila* in cancer research: the first fifty tumor suppressor genes. J. Cell Sci. **18** (Suppl): 19–33.

Weinkove, D., T. P. Neufeld, T. Twardzik, M. D. Waterfield and S. J. Leevers, 1999 Regulation of imaginal disc cell size, cell number and organ size by *Drosophila* class I(A) phosphoinositide 3-kinase and its adaptor. Curr. Biol. **9:** 1019–1029.

Wilkin, M. B., M. N. Becker, D. Mulvey, I. Phan, A. Chao *et al.*, 2000 *Drosophila* dumpy is a gigantic extracellular protein required to maintain tension at epidermal-cuticle attachment sites. Curr. Biol. **10:** 559–567.

Wu, S., J. Huang, J. Dong and D. Pan, 2003 *hippo* encodes a Ste-20 family protein kinase that restricts cell proliferation and promotes apoptosis in conjunction with *salvador* and *warts*. Cell **114:** 445–456.

Xu, T., W. Wang, S. Zhang, R. A. Stewart and W. Yu, 1995 Identifying tumor suppressors in genetic mosaics: the *Drosophila lats* gene encodes a putative protein kinase. Development **121:** 1053–1063.

Yarunin, A., V. G. Panse, E. Petfalski, C. Dez, D. Tollervey *et al.*, 2005 Functional link between ribosome formation and biogenesis of iron-sulfur proteins. EMBO J. **24:** 580–588.

Zhang, H., J. P. Stallock, J. C. Ng, C. Reinhard and T. P. Neufeld, 2000 Regulation of cellular growth by the *Drosophila* target of rapamycin dTOR. Genes Dev. **14:** 2712–2724.

Zhou, Z., L. J. Licklider, S. P. Gygi and R. Reed, 2002 Comprehensive proteomic analysis of the human spliceosome. Nature **419:** 182–185.

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