Cell-autonomous and non-autonomous growth-defective mutants of

Drosophila melanogaster

Mireille Galloni and Bruce A. Edgar

Fred Hutchinson Cancer Research Center, Division of Basic Sciences, B2-152, 1100 Fairview Ave N., Seattle, Washington, 98109, USA

(e-mails: mgalloni@fred.fhcrc.org; bedgar@fred.fhcrc.org)

SUMMARY

During animal development, growth of the various tissues and organs that make up the body must be coordinated. Despite recent progress in understanding growth control within the cell unit, the mechanisms that coordinate growth at the organismal level are still poorly understood. To study this problem, we performed a genetic screen for larval growth-defective mutants in *Drosophila melanogaster*. Characterization of these mutants revealed distinct types of larval growth defects. An allelic series for the translation initiation factor, Eif4A, showed different growth rates and suggests that Eif4A could be used as a dose-dependent growth regulator. Two mutants that fail to exit cellular quiescence at larval hatching (*milou* and *eif4A^{1006}*) have a DNA replication block that can be bypassed by overexpression of the E2F transcription factor. A mutation (*bonsaï*) in a homolog of the prokaryotic ribosomal protein, RPS15, causes a growth defect that is non-cell-autonomous. Our results emphasize the importance of translational regulation for the exit from quiescence. They suggest that the level of protein synthesis required for cell cycle progression varies according to tissue type. The isolation of non-cell-autonomous larval growth-defective mutants suggests that specialized organs coordinate growth throughout the animal and provides new tools for studies of organismal growth regulation.

Key words: *Drosophila*, Larval growth defect, Organismal growth, *eif4A*, Protein synthesis, Non-autonomy

INTRODUCTION

The 'correlation of growth' between the different parts of a multicellular organism is an old observation (Darwin, 1859). Despite the complexity and diversity of growth modes for different tissues and cells, there is a remarkable growth coordination that takes place to give rise to and to maintain a normal individual. In species where individuals exhibit natural size variations, like humans, proportions between the different organs and cell composition within the same tissues are maintained according to the individual's size. Tissue size and identity are also maintained throughout development and adult life. Different tissues and cell types utilize different modes of growth: increase in cell mass, in cell number, in DNA ploidy, symmetric and asymmetric cell divisions, etc. These growth modes change between embryonic development and adult life, and they can also be modulated according to environmental and physiological conditions (e.g. food availability, temperature, pregnancy, etc.). Higher order controls on cell proliferation must exist that ensure growth coordination and synchronization of multiple tissues within a single individual, yet the nature of these controls remains essentially unexplored. What coordinates and controls the growth of different organs and what is the genetic basis for tissue and organism homeostasis?

The *Drosophila* larva presents a unique system to study the control of growth and cell cycle initiation in the context of a whole organism. Different cell cycles exhibiting different types of control occur in different tissues during *Drosophila* development (for review see Edgar and Lehner, 1996). During the embryonic cycles, DNA replication is initiated immediately after mitosis: cell-cycle regulation occurs at the transition to mitosis. At the end of embryogenesis, most cells have stopped proliferating and have entered into an extended quiescent phase (G_1 or G_0) from which they will be released after hatching and feeding of the larvae. Most tissues necessary for larval life exhibit a type of cell cycle called endocycle in which cells undergo several rounds of DNA replication without dividing (Smith and Orr-Weaver, 1991). These endocycles are among the first in *Drosophila* development that are regulated at the initiation of DNA replication. During larval life, a period devoted to growth, a 200-fold increase in mass (Church, 1965) occurs in 4 days by increases in cell size rather than by an increase in cell numbers. The high degree of polyteny resulting from several rounds of regulated endocycles is most likely required for the extensive cell growth observed in larval tissues. Therefore, failure to start endoreplication of the DNA should result in larval growth defects (LGD) without otherwise impairing larval life (Royzman et al., 1997). We predicted that at least some LGD mutants should also exhibit defects in DNA replication, and that an extended larval survival would indicate an absence of general metabolic defects in the mutants. Based on these hypotheses, we screened for larval growth-defective

Drosophila mutants with an extended larval life and assessed whether they could initiate the cell cycle. To search for genes potentially involved in growth signalling, we analyzed mosaic mutant animals to identify non-cell-autonomous functions. Here we describe and compare nine mutant lines that exhibit different growth and DNA replication defects, longevity and cell-autonomy phenotypes.

MATERIALS AND METHODS

Genetic screens

Primary screens: two cultures/line of ~100 larvae fed on fresh yeast (Fleischmann) were checked. Discrimination of homozygous mutant larvae from their siblings was based on size differences at 4 days after hatching (AH). Lines with ~25% of small larvae with normal morphology and viability were selected. Preliminary larval stages were determined on the basis of size difference between L1 and L2, and of anterior spiracle morphology for L2 and L3 (Bodenstein, 1950). For identification of mutant larvae from 2nd chromosome lines, we used a *CyO,P[y⁺]* balancer in a *yw* background. *yw;∗/CyO,P[y⁺]* stocks were established from *yw;∗/CyO × yw;R21/CyO,P[y⁺]* crosses raised at 18°C, the restrictive temperature for *R21* (*R21=Fs(2)ketel¹R21*, Török et al., 1993; ∗=mutant chromosome). *y* mutant homozygotes have lighter colored mouth hooks than *y⁺* heterozygotes. Longevity and growth of mutant larvae: typically 4 cultures/lines of ~200 embryos were set at 25°C in instant fly food (Carolina Biological Supply Company)/fresh yeast vials. Larval population was first checked 4 days AH, then typically 8 days, 10 days and 12 days AH or until no more small larvae were found. ~50 *y* larvae were expected to be found/vial: mutant larvae viability is expressed in % of maximum number of homozygotes (Table 1).

Fly stocks, reversion experiments and complementation tests

3013 P-element-induced recessive lethal lines were provided by the Berkeley *Drosophila* Genome Project (BDGP) and the Bloomington Center (2544, 467 and 2 lines on the 2nd, 3rd and 4th chromosome, respectively). Most mutant lines carried the *P[lacW]* transposon (Bier et al., 1989). Reversion crosses: *yw;[]/CyO,P[y⁺]* revertant males (white eyes; *[] = P[w⁺]* excision) were recovered from *yw;P[w⁺]/CyO;Δ2,3,Sb/+* males × *yw;R21/CyO,P[y⁺]* or *yw;P[w⁺]/CyOP[y⁺]* females of the same mutant line. 10 revertant males/mutant were crossed independently to *yw;R21/CyO,P[y⁺]* females to establish stocks. Lines where *CyO⁺,y,w* progeny were found were scored as true revertants, lines for the same mutants for which only *CyO,y⁺,w* progeny were found were scored as potential new alleles. In the cases where the P marker was lost but where no true revertant was found, existence of a background lethal mutation was suspected. For *milou*, this was proven by separating the lethal mutation and *P[w⁺]* by meiotic recombination and establishing a *w milou* line that retained the original LGD phenotype. *milou*'s location was deduced from non-complementation of *Df(2R)Px²* and complementation of *Df(2R)Px⁴*. Other deficiency lines used were: *Df(2L)GpdhA* and *Df(2L)cl-h3* for *eif4A*, *Df(2L)TW50* for *plume*, *Df(2R)vg-C/SM5* and *Df(2R)vg135* for *poney*, *Df(2R)59AB* for *colibri*. *bonsaï* location was inferred from BDGP chromosomal mapping of P1 phage AC004377. Complementation tests: in unidirectional crosses, when no *CyO⁺* F₁ flies but at least 100 *CyO* F₁ were counted, the parental lines were found to belong to the same complementation group.

Plasmid rescue and genomic DNA analysis

Genomic DNA flanking both sides of the P insertion site was recovered by plasmid rescue and sequenced as described (Pirrotta, 1986; Bier et al., 1989). Sequences homologies were searched in the database by using the BLAST program (Altschul et al., 1990).

DNA labelling

In vivo DNA labelling experiments were performed as described (Britton and Edgar, 1998). Samples were visualized with bright-field Nomarski and UV fluorescent microscopy.

E2F/DP bypass experiments

Heat-shock (HS) inducible *HS-E2F* and *HS-DP* cDNAs transgenes (Duronio et al., 1995) were introduced into the different mutant backgrounds (∗) by crossing *yw;∗/CyO,P[y⁺]* mutants with a *yw;Sp/CyO,P[y⁺];P[w⁺,HS-E2F],P[w⁺,HS-DP]/TM3Sb* line. Stocks of the genotype *yw;∗/Cy,P[y⁺];P[w⁺,HS-E2F],P[w⁺,HS-DP]* were established. Egg collections were performed for ~4 hours. Eggs were dechorionated (2% bleach) and ~200 eggs/culture were placed either in sucrose medium (20% sucrose in PBS) or in instant fly food/yeast extract. After 3 days, the sucrose and normal food cultures were heat shocked for 2 hours at 37°C followed by addition of BrdU (100 µg/ml). Cultures recovered for >8 hours at room temperature prior to dissection. Immunodetection was performed as described above. Mutant homozygotes (*y*) were distinguished from heterozygote siblings (*y⁺*) based on mouth hook color.
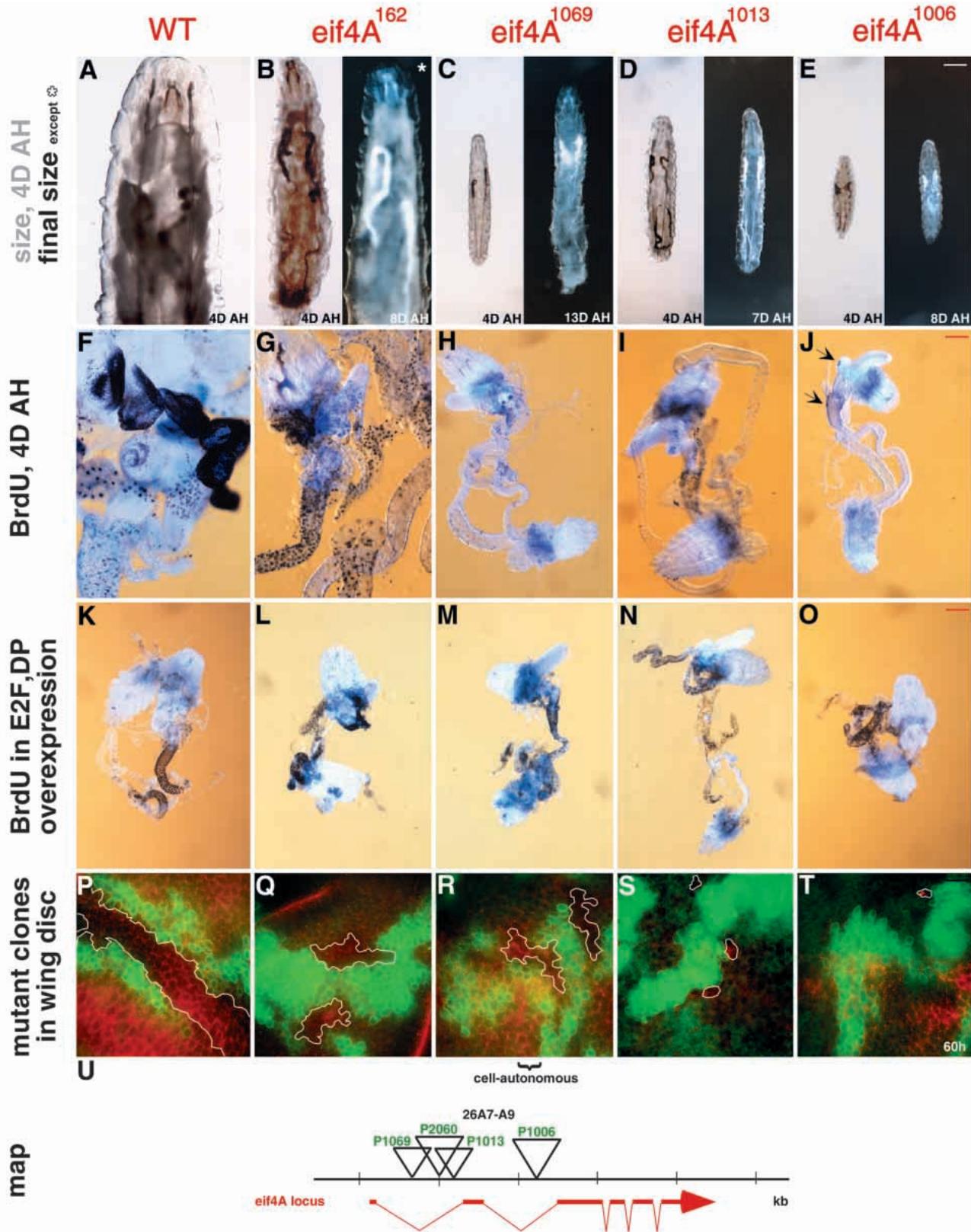
Clonal analysis

Genetic mosaics were made by using FLP/FRT mitotic recombination (Golic and Lindquist, 1989; Xu and Rubin, 1993). The different mutations were recombined onto chromosomes carrying an FRT site at cytological position 40A for *eif4A¹⁰⁰⁶*, *eif4A¹⁰¹³*, *eif4A¹⁰⁶⁹* and *eif4A¹⁶²* and at 42D for *milou*, *bonsaï*, *poney* and *colibri*. *yw* or *w;FRT40A or 42D,∗/CyO,P[y⁺]* males were crossed to *yFLP¹²²;Sp/SM6-TM6Tb* females, and *yFLP¹²²;FRT40A* or *42D,∗/SM6-TM6Tb* F₁ males were crossed to *w;FRT40A,P[w⁺,HS-Nmyc]* or *w;FRT42D,P[w⁺,HS-πmyc]* females. 4 hours egg collections were heat shocked for 1 hour at 37°C, typically ~36-40 hours AH, than returned at room temperature for 2 days. *Tb⁺* female larvae were dissected in PBS ~94-96 hours AH, after a 1 hour HS at 37°C to allow expression of the *myc* marker. Samples were fixed and stained as described (Xu and Rubin, 1993). Discs were observed by Deltavision microscopy. Because after several attempts *plume*,FRT40A recombinants were not recovered, *plume* clones were not investigated. Mosaic eyes were obtained by inducing clones with the eye-specific Eyeless-Flipase (*ywEyFLP;Sp/CyO,y⁺*, B. Dickson, personal communication): mutants of the genotype *ywEyFLP;FRT40A* or *42D,∗P[w⁺]/CyO,y⁺* were crossed to *ywEyFLP; FRT40A* or *42D/CyO,y⁺* lines. *eif4A¹⁶²* and *milou* that lack *P[w⁺]* were crossed to *ywEyFLP;FRT40A,P[w⁺,HS-πmyc]* or *FRT42D,P[w⁺,HS-Nmyc]/CyO,y⁺*. Flies of the same age with and without clones were compared.

RESULTS

A genetic screen for larval growth-defective phenotypes

After embryo hatching (AH), *Drosophila* larval development spans about 4 days at 25°C and includes three larval instars: L1, one day, L2, one day and L3, two days. The mature L3 larva has at least 200-times more mass than the newly hatched L1 (Church, 1965; see Fig. 2A,B). Our screens for larval growth defects (LGD) were based on the isolation of mutant lines that do not reach the normal L3 size. Results are summarized in Fig. 1. In a primary screen, 3013 existing P-element-induced recessive lethal mutants were scored for LGD phenotypes at 4 days AH. Many lines (870) had an abnormal larval subpopulation exhibiting various types of defects, including 393 lines showing some degree of larval growth retardation. Out of these, we retained 100 lines that had a

3013 P element-induced recessive lethal lines
(2544 2nd chromosome, 467 3rd chromosome, 2 4th chromosome)

↓

870 abnormal larval population

↓

393 various larval growth defects

↓

100 LGD with normal morphology, viability and behavior at 4 days AH
(84 2nd chromosome, 16 3rd chromosome)

↓

84 LGD 2nd chromosome
(L1-, L2- and L3-arrests and growth delays)

↓ ↓

21 4 days< longevity <8 days   **63** longevity > 8 days

↓ ↓

max. **17** complementation groups   **21** complementation groups

↓ ↓ ↓

colibri   **eif4A**   **milou**
                                        plume
                                        poney
                                        bonsai

Fig. 1. Genetic screens for growth-defective mutants. 3013 P-element-induced recessive lethal lines were screened for larval growth defects (LGD) 4 days AH. 870 lines showed abnormal larval phenotypes including various LGD, clear fat body, duplicated mouth hooks, tumors, etc. 100 LGD lines with normal morphology were selected. Out of these, 84 lines on the 2nd chromosome were screened for larval longevity and nature of the growth defect (arrests or delays). Several mutants representative of the LGD collection with an extended survival were chosen for further analysis: DNA labelling, E2F/DP bypass and mosaic analysis(*eif4A* alleles, *bonsaï*, *poney*, *plume* and *milou*). The low survival mutant *colibri* was followed for comparison with the other mutants.

revealed that the 2nd chromosome LGD lines with a larval survival of more than 8 days AH fall into 21 complementation groups. Ten of these groups were not considered further because they carried background lethal mutations, multiple P elements or other problems (representative lines: *l(2)k04907*, *l(2)k05103*, *l(2)k05918*, *l(2)k06131*, *l(2)k09315*, *l(2)k10613*, *l(2)k11006*, *l(2)k13104*, *l(2)k16918*, *l(2)01296*; BDGP; our data). Of the 11 remaining complementation groups, 9 contained a single allele, e.g. *bonsaï*, *poney* and *milou*. One group, *plume*, was represented by three lines exhibiting essentially the same phenotype. Finally, one group had 7 members and was found to be an allelic series of the *eif4A* locus including the original *l(2L)162^{neo}* mutation (Dorn et al.,

subpopulation of abnormally small larvae with normal morphology, feeding and behavior (84 lines on the 2nd chomosome and 16 lines on the 3rd chromosome). These lines could be divided into four classes according to the size of the mutants: (1) L1 size, (2) L2 size, (3) small L3 size and (4) variable size.

We focused on the 84 2nd chromosome lines that were subsequenlty screened for extended survival of the mutant homozygote larvae. Longevity of at least 8 days AH was sought since wild-type larvae deprived of dietary proteins but provided with an energy source (sucrose) are able to survive until that time (Britton and Edgar, 1998). Larvae raised on nutrient-free agar do not grow, and die in 2 or 3 days (not shown; see Fig.2A). In this secondary screen, 63 lines were found to have a subset of the mutant population surviving more than 8 days AH, whereas 21 lines had a larval longevity of 4-8 days AH. Larval size was scored throughout the longevity assay: several types of larval growth arrests and growth delays were observed. In growth delay phenotypes, two classes were found: larvae that grew slowly but eventually reached the mature size, and larvae that arrested at a reduced L3 size. Thus, we distinguished different types of growth defects that could be indicative of different types of impaired growth functions. In mutant larvae, no relative imaginal disc overgrowth was detected. The size of discs in mutant larvae appeared proportional to larval size. Our screens did not reach saturation, but large-scale screens for EMS-induced LGD mutants are likely to cover most of the larval growth loci for the 2nd chromosome (M. S. Garfinkel and B. A. Edgar, unpublished data). Other LGD mutants have also been characterized (for example, Qu et al., 1997; The et al., 1997; Zaffran et al., 1998; Migeon et al., 1999; S. A. Datar and B. A. Edgar, unpublished data).

The number of loci represented in our mutant collection was determined by complementation analysis. These tests

WT larval size   **WT BrdU incorporation**

Fig. 2. Growth and DNA replication in wild-type larvae. (A,B) Whole-mounted larvae, anterior to top. (A) Newly hatched L1 larva, 0 days AH. (B) Mature L3 larva, 4 days AH. (C-E) Dissected larvae with DNA labelling by BrdU incorporation (black dots) during 24 hours periods from hatching (C), 1 day AH (D), 3 days AH (E). Final age of the larvae is indicated in each case. Anterior to left, dorsal to top. Note that Figs 2A,B, 3A-E, 4A-E and 2C-E, 3F-O, 4F-O are at the same magnification.

Figs 3, 4. Larval growth defect mutants. Figs 2A,B, 3A-E and 4A-E, 2C-E, 3F-O and 4F-O, and 3P-T and 4P-T are at same magnifications, respectively. Anterior to the top, unless noted. (3A-E, 4A-E) Larval size and longevity, whole-mounted larvae. Left (light panels), larval size 4 days AH; right (dark panels), final size and longevity in days. Note that in wild-type (3A), only anterior half of the larva is visible. (3B*) *eif4A^162* final size, ~11 days AH, close to mature L3. (4A*) *bonsaï* final size, ~20 days AH, small flies. (3F-J, 4F-J) DNA synthesis on normal diet: larvae were dissected 4 days AH after a 24 hours labelling period with BrdU. (3K-O, 4K-O) DNA synthesis after E2F-DP overexpression on sucrose diet: larvae were dissected a few hours after E2F-DP induction. (3P-T, 4P-T) Mutant clones in imaginal wing discs.
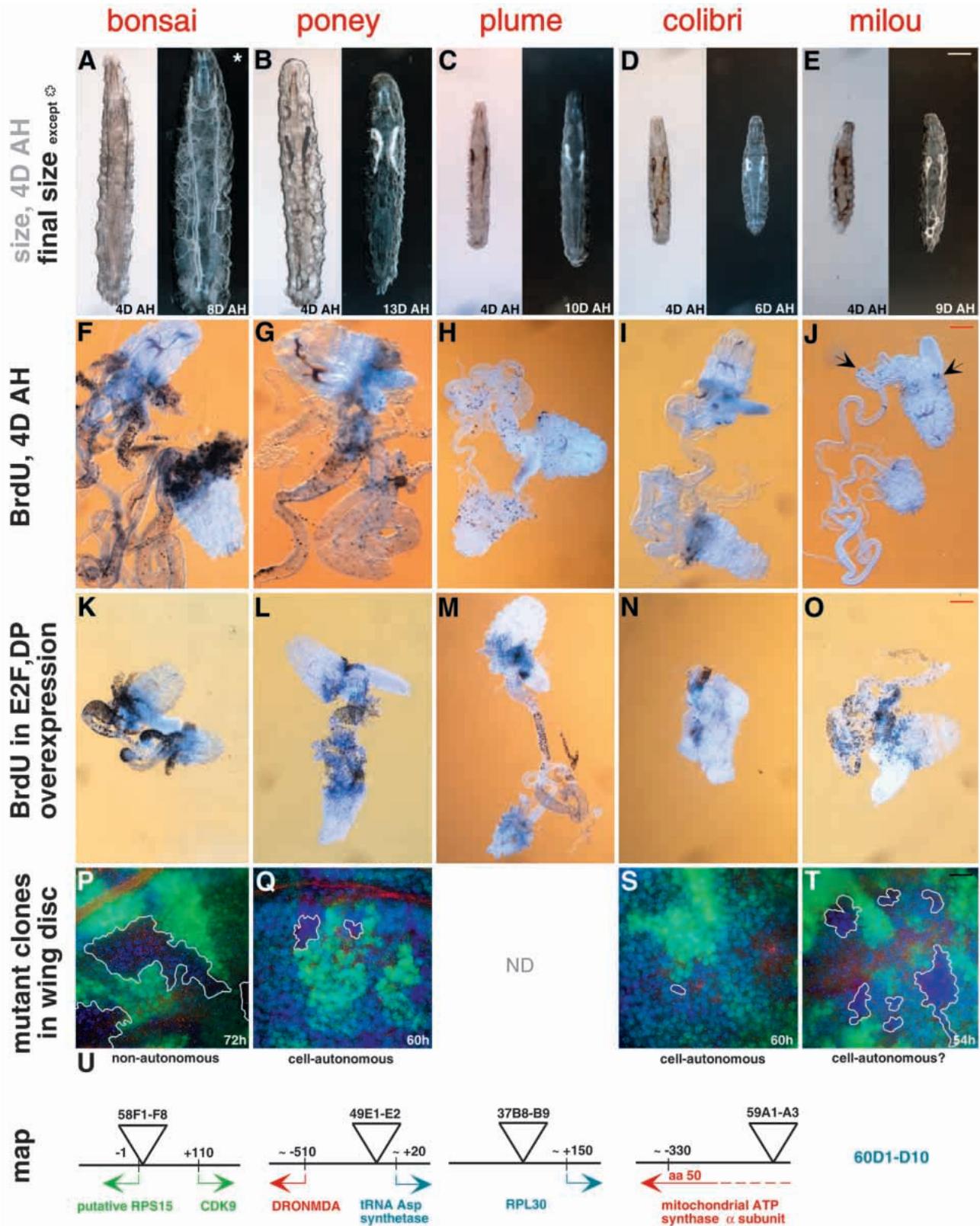
Wing pouch region, magnification ×630. Clone age is indicated in hours (H) AH (lower right). Nuclei, blue; actin, red. Heterozygous cells express one dose of myc marker (pale green), either in the plasma membrane (*eif4A* alleles) or in the nucleus (*bonsaï, poney, colibri* and *milou*). Mutant clones are unmarked and wild-type twin clones have two doses of marker (bright green). Mutant clones are outlined in white. A wild-type unmarked clone is shown in Fig. 3P. (3U, 4U) Map**:** P element (triangle) position relative to transcription units (TU) is shown in base pairs (bp). Existing TU, red; novel TU, green; potential TU, blue bottom lettering. Chromosomal location obtained from the BDGP is indicated above the P element (top, black lettering).

Table 1. Mutant phenotypes and molecular information

Mutants	LGD Phenotype	Survival and longevity	DNA replication	Cell autonomy	P-element position and chromosomal location
wild-type, nutrient-free	LGA L1	3 days AH	for a few hours AH	x	x
wild-type, sucrose-fed	LGA L1	8 days AH	MNBs, G	x	x
eif4A^{162} l(2)02439	LGDL L3	45% at 5 days AH 4% at 10 days AH	+	yes	1st intron of *eif4A* 26A-B
eif4A^{1069} l(2)k15403	LGA L2	60% at 4 days AH 42% at 8 days AH	+/−	yes	1st intron of *eif4A* 26A-B
eif4A^{1013} l(2)k14610	LGA L1	28% at 4 days AH	+/−	yes	1st intron of *eif4A* 26A-B
eif4A^{1006} l(2)k14518	LGA L1	60% at 6 days AH 10% at 8 days AH	−	yes	2nd intron of *eif4A* 26A-B
bonsaï l(2)k08322	LGR L3 and small flies	100% at 8 days AH 50% at 11 days AH 8% at 13 days AH	+	no	1 bp 5′ of RPS15 homolog and 110 bp 5′ of CDK9 58F1-F8
poney l(2)k05411 (l(2)k05408)	LGA L2 and L3 same size	60% at 4 days AH 25% at 11 days AH	+/−	yes	5′ of aspartyl-tRNA synthase gene 49E1-E2
plume l(2)k13302 l(2)k13307, l(2)k00308)	LGA L1	70% at 4 days AH 20% at 8 days AH	+/−	nd	5′ of a putative RPL30 37B8-B9
colibri l(2)k04409	LGA L1	20% at 4 days AH 16% at 5 days AH	+/−	yes	intron of mit. ATP synthase α subunit 59A1-A3
milou l(2)k13910	LGA L1	60% at 4 days AH 10% at 9 days AH	−	?	60D1-D10*

Mutant names, bold; original names, plain text; other alleles, (); MNB, mushroom body neuroblasts; G, gonad.
LGD, LGA, LGR, LGDL phenotypes: Larval Growth Defect, Arrest, Reduced, Delayed. L1, L2, L3: 1st, 2nd and 3rd instars.
Survival is expressed in % of expected total number of homozygotes. Longevity, in days after hatching (AH). Chromosomal location, BDGP except *.

1993; BDGP) here referred to as *eif4A^{162}*. Including 3 alleles with a survival of less than 8 days and a growth delay mutant, 11 alleles of *eif4A* with different phenotypes were found in our LGD screens. 4 alleles representative of this series are described here: *eif4A^{1006}*, *eif4A^{1013}*, *eif4A^{1069}* and *eif4A^{162}* (other alleles: *l(2)k05206, l(2)k07218, l(2)k07519, l(2)k08120, l(2)k09234* and *l(2)k16006, l(2)k16010*).

To assess whether the P-element insertion was responsible for the mutant phenotypes, reversion experiments were attempted on at least one member of each complementation group. Transposon excision was scored in dysgenic flies by loss of the *P[lacW] miniwhite$^+$* eye color marker (for *eif4A^{162}*, see Dorn et al., 1993). Reversion of the recessive lethality was then checked in P excised homozygotes. In all cases, *white* progeny were recovered from these crosses, but in 7 cases out of 11 reversion of lethality was not observed (*l(2)k02405, l(2)k02503, l(2)k04512, l(2)k05224, l(2)k05433, l(2)k13910* (*milou*), *l(2)k14608*). In these lines, a second site mutation rather than the P element might be responsible for the mutant phenotypes. Mutant lines bearing mutations that could be reverted by P-element excision and which presented a comprehensive range of phenotypes were selected for further characterization: *eif4A^{1006}*, *eif4A^{1013}*, *eif4A^{1069}* and *eif4A^{162}*, *bonsaï*, *poney* and *plume*. In addition, the *milou* mutant, which was not linked to the P element, was also chosen because it exhibits a unique combination of phenotypes. For comparison purposes, one revertable mutant with a 6 day survival, *colibri*, was also retained for further analysis. Growth and longevity of

the mutants are presented in Figs 3A-E, 4A-E (for comparison with wild-type larvae, see Fig. 2A,B). Details of the mutant phenotypes are given in Table 1.

Molecular analysis

Identification of the affected genes in the mutant lines was initiated by analysis of the genomic DNA flanking the P insertion site on both sides. In all cases, highly significant homologies to known genes were found. In two instances, the locus affected in the mutants was clearly identified: *eif4A*, which was already known and *bonsaï*, for which we obtained rescue of the LGD phenotype with wild-type transgenes (data not shown). *eif4A^{1013}*, *eif4A^{1069}*, *eif4A^{162}* and *eif4A^{1006}* P elements are inserted into the first and second introns of *eif4A*, respectively. In *bonsaï*, the P element prevents transcription of a 280 amino acid putative coding region. The 90 amino acids homologous to the entire prokaryotic ribosomal protein (RP) S15 are embedded in the middle of this open reading frame. In *poney*, the transposon is inserted close to a putative aspartyl-tRNA synthase gene and to the DRONMDA locus (N-methyl-D-aspartate receptor-associated protein, Pellicena-Palle and Salz, 1995). The *plume* P element is likely to be inserted 5′ of a putative RP L30 at position 37B8-B9 (BDGP; our data). In *colibri*, the P element is inserted into the first intron of the α subunit of a mitochondrial ATP synthase homolog (Talamillo et al., 1998). The chromosomal locations obtained from the BDGP were confirmed by non-complementation of mutant lines bearing deletions for the different P insertion sites.

Finally, the location of the *milou* mutation at 60D1-D10 was inferred from complementation analysis with 2nd chromosome deficiency lines. Thus many of the mutants recovered are potentially defective in protein synthesis-related functions. These results are reported in Figs 3U and 4U.

Larval growth defects and DNA replication

Larval growth most likely requires an increase in DNA ploidy of the larval cells, but failure to replicate the DNA should not impair larval life (Royzman et al., 1997). To look for mutants unable to exit from quiescence, we tested whether the growth defects in our mutant lines were accompanied by DNA replication defects. BrdU was fed for 24 hours to 3-day-old larvae from 39 lines (15 complementation groups) and incorporation into DNA was visually compared between 4-day-old homozygote and heterozygote siblings (similar to wild-type controls). All mutants appeared to feed since colored food was detected in the gut (not shown). Only two mutants exhibited a near absence of DNA replication: *eif4A^{1006}* and *milou* (Figs 3F-J, 4F-J; for wild-type, see also Fig. 2C-E). A few nuclei throughout the body, in particular the mushroom body neuroblasts (MNBs), were still able to replicate DNA in these mutants (see arrows in Figs 3J, 4J), for as long as 9 days AH in *milou* larvae. These mutants therefore behave like protein-deprived wild-type larvae (Britton and Edgar, 1998). The *eif4A^{1013}*, *eif4A^{1069}*, *poney*, *plume* and *colibri* mutants showed reduced levels of DNA replication. Finally, in the less-severe LGD mutants *eif4A^{162}* and *bonsaï*, BrdU incorporation was similar to wild type. In *bonsaï* brains, however, cell proliferation was impaired as compared to other tissues and to wild-type (Fig. 5A,B). Although *colibri* larvae grew less than *milou* larvae, DNA replication was stronger in *colibri* than in *milou* mutants. Thus, absence of DNA replication was rare and seemed to be observed only in some of the most severe LGD mutants.

Overexpressing E2F activates DNA replication in the LGD mutants

When cultured on a sucrose-only medium, wild-type larvae can survive for 8 days but do not initiate DNA replication and grow very little. Only the MNBs and gonads sustain DNA replication under these conditions. The block to replication in the other tissues is alleviated when amino acids are added to the sucrose medium, or when the cell-cycle-related transcription factor E2F/DP is overexpressed in starved larvae (Britton and Edgar, 1998). To check whether the DNA replication defects in the LGD mutants could be rescued, we induced overexpression of E2F/DP in the different mutant backgrounds (Figs 3K-O, 4K-O). This experiment indicated which gene functions were epistatic to E2F/DP in the initiation of DNA replication. Upstream gene functions were expected to be bypassed by E2F/DP overexpression whereas downstream functions were not. Furthermore, this test could also indicate whether protein synthesis was impaired in the mutants, since the E2F/DP transcription factor and at least some S phase proteins must be translated to have an effect.

Surprisingly, BrdU incorporation was greatly induced when E2F/DP was overexpressed by heat shock (HS) in *milou* and *eif4A^{1006}* larvae even when starved (Figs 3O and 4O, compare with BrdU normal-fed larvae without HS-E2F/DP, Figs 3J and

4J). This indicated that the loss of DNA replication in these mutants could be bypassed and that protein synthesis was not completely abolished. Likewise, E2F/DP overexpression also induced DNA replication in all other mutants under starvation conditions (Figs 3L-N and 4L-N).

Not all growth defects are cell autonomous

To distinguish between cell-autonomous and non-cell-autonomous functions, clonal analyses of homozygous mutant cells in a wild-type background were performed. Cell-autonomous growth defects are expected to give rise to cells unable to proliferate, or to clones that grow more slowly than normal. Slow growing cells should be eliminated and replaced by wild-type cells, such as in the case of *Minute* mutations (Morata and Ripoll, 1975; Simpson, 1979). On the contrary, non-cell-autonomous mutations should give rise to wild-type size mutant clones that are maintained throughout development. Clones of mutant cells were followed in third instar larval imaginal discs. We focused on wing discs as shown in Figs 3P-T and 4P-T, but essentially similar results were obtained in other discs. Mutant clones were compared to their wild-type twin clones induced at the same time. Mosaic adults were compared to adults of the same genotype without clones and were checked for phenotypic defects throughout the body.

eif4A^{1006} mutant clones were virtually undetectable; only occasionally one mutant cell was visible close to the wild-type twin spot (Fig. 3T). The detection of a few mutant cells was slightly more frequent with *eif4A^{1013}* (Fig. 3S). Larger clones were found with *eif4A^{1069}*, and even larger ones with *eif4A^{162}* (Fig. 3Q-R), but they never reached twin spot size (compare also with unlabelled wild-type clone, Fig. 3P). This showed that *eif4A* mutations behaved cell autonomously. All adults in which *eif4A^{1006}* and *eif4A^{1013}* clones had been induced showed a rough eye phenotype (Fig. 5C,D), which was not visible with the weakest alleles *eif4A^{1069}* and *eif4A^{162}*. Other adult tissues were virtually normal; only in rare cases a small cut was detected in adult wings. This suggested that not all tissues responded similarly to *eif4A* mutations.

poney and *colibri* mutant clones also showed a severe size reduction, arguing that these gene products are required for cell proliferation in a cell-autonomous manner (Fig. 4Q-R). Mosaic adults looked normal. *milou* homozygote mutant cells were able to proliferate and could produce clones close to wild-type size when induced at 42 hours AH (Fig. 4T). However, *milou* clones were smaller than twin-spots when induced earlier (24 and 36 hours AH), indicating that *milou* cells exhibit some degree of growth defect. Adult wings in which *milou* clones had been induced frequently showed large cuts along the entire wing margin that were not found in progeny of the same genotype with no clone induction (Fig. 5E,F). This suggested that *milou* cells were able to proliferate for long periods of time and were eliminated after pupariation.

Finally, *bonsaï* clones induced at 24 (Fig. 4P), 36 and 42 hours AH grew to a size similar to wild type. No defects were observed in mosaic adults, indicating that the mutant cells grew normally and were not eliminated during development. Thus, *bonsaï* mutant cell growth is rescued in a wild-type environment. This could be due to the fact that *bonsaï* is not required in imaginal discs or, alternatively, that the Bonsaï product has a non-cell-autonomous function.
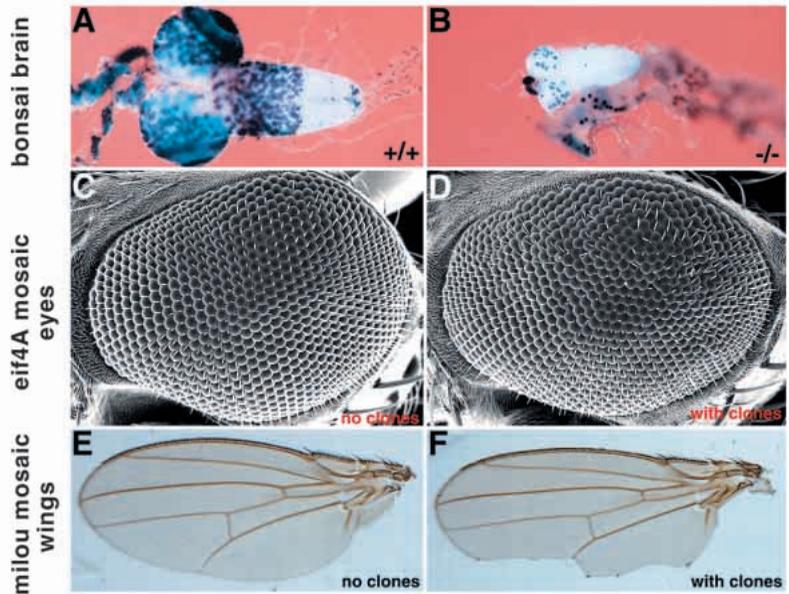
Fig. 5. Different tissues are affected differently in the mutants. (A,B) DNA labelling (black dots) in wild-type (A) and *bonsaï* (B) brains. Anterior to left. A proliferation defect is detectable in *bonsaï* brains but not in other mutant tissues. (C-F) *eif4A* and *milou* mosaic flies. Random clones of homozygous mutant cells were induced during the larval period in heterozygous animals. (C,D) SEM of adult compound eyes, anterior to bottom. Eyes in which *eif4A* clones have been induced are rough (D) as compared to eyes of the same genotype without clones (D). Other tissues in the adults are normal. (E,F) Whole mounted wings with *milou* clones (F) frequently have large cuts not seen in wings without clones (E). Anterior to top.

Not all tissues are affected similarly in the mutants

In the experiments described above, clones were not labelled with adult markers. We took advantage of the fact that most mutations were due to *P[mw+]* insertions (*mw+= miniwhite+*) to examine the behavior of marked mutant clones in adult eyes (for *milou* and *eif4A^{162}*, mutant clones are *white*). These clones were induced very early in development in the eye anlagen by the use of an eye-specific Flipase (Eyeless-Flipase, B. Dickson, personal communication; *eyeless*: Quiring et al., 1994).

In mosaic eyes, *eif4A* mutant clones never reached wild-type size and grew according to the allele's strength: the stronger the allele the smaller the clones (Fig. 6A-H). This is in agreement with the gradation of other phenotypes that we observed. In most cases, particularly with *eif4A^{1006}* and *eif4A^{1013}*, we detected rough eye phenotypes, confirming the results obtained in the mosaic animals described above. The strongest rough eye phenotypes seem to correlate with the least number of mutant cells since, in *eif4A^{1006}* and *eif4A^{1013}* mosaic eyes, almost no mutant cells are detected. This apparent non-autonomous effect could be due for instance to impaired Notch signalling since *eif4A* mutations enhance *Notch* eye phenotypes (Röttgen et al., 1998).

In *milou* mosaic eyes, very few mutant cells were detected: these eyes appear to be mostly composed of wild-type ommatidia (Fig. 6O,P). As in wing discs, *milou* clones in imaginal eye discs were able to produce large clones, albeit not of wild-type size. Abundant small compacted nuclei could be detected by DAPI staining at the site of these clones, indicating that mutant cells may undergo apoptosis (data not shown). Indeed, increased cell death was observed by acridine orange staining in *milou* mosaic eyes as compared to wild-type (not shown). This suggests that, although *milou* cells can proliferate, they do not survive, a result in agreement with cut wings found in mosaic animals (Fig. 5F).

In contrast to clones in wing discs and to their LGD phenotypes, *poney* and *colibri* clones were readily detectable in mosaic eyes, as visible by their variegated phenotype (Fig. 6K-N). These eyes are made of dark red mutant clones (*mw+/mw+*) and *white* wild-type twins, whereas heterozygote eyes (*mw+/+*)

have a uniform orange color. Therefore, *poney* and *colibri* cells seem to grow better in the eye than in the wing disc. This could reflect tissue-specific differences in proliferation rates that were previously reported (Fain and Stevens, 1982).

Finally, like mosaic wing discs, *bonsaï* mosaic eyes were found to be composed of two types of clones of equal proportions: *white* wild-type and dark red *bonsaï* clones (Fig. 6I,J). Therefore, we conclude that *bonsaï* cells proliferate and survive in adult animals like wild-type cells, a strong argument in favor of non-autonomy for the Bonsaï product.

DISCUSSION

An allelic series suggests that Eif4A levels could regulate growth

Eif4A is an ATP-dependent RNA helicase that is an essential part of the Eif4F translation initiation factor like Eif4E and Eif4G. Translation initiation is the most commonly regulated step in translation (Mathews et al., 1996). Eif4F binds to mRNAs and allows assembly with the small ribosomal subunit. Eif4A interacts with both mRNA ends (Craig et al., 1998) and is thought to promote unwinding of complex 5′ untranslated regions (UTRs) and thereby enhance ribosome binding (Merrick and Hershey, 1996). We have recovered several mutant alleles of *eif4A* that exhibit a gradation of larval growth, DNA replication defects and mutant clone size and which can be ordered according to the severity of the phenotypes: *eif4A^{1006}*>*eif4A^{1013}*>*eif4A^{1069}*>*eif4A^{162}*. Since three *eif4A* alleles that show a wide range of defects are clustered within the first intron of the gene (Fig. 3U), quantitative differences in Eif4A levels probably cause the mutant phenotypes. In the most severe *eif4A^{1006}* mutant in which growth and DNA synthesis are almost abolished, high levels of DNA replication can be induced by overexpressing the E2F/DP transcription factor (compare in Fig. 3J and 3O). This shows that DNA and protein synthesis can occur in this mutant and that its defects lie upstream of E2F/DP for the activation of DNA replication. This suggests that a translation hierarchy exists between
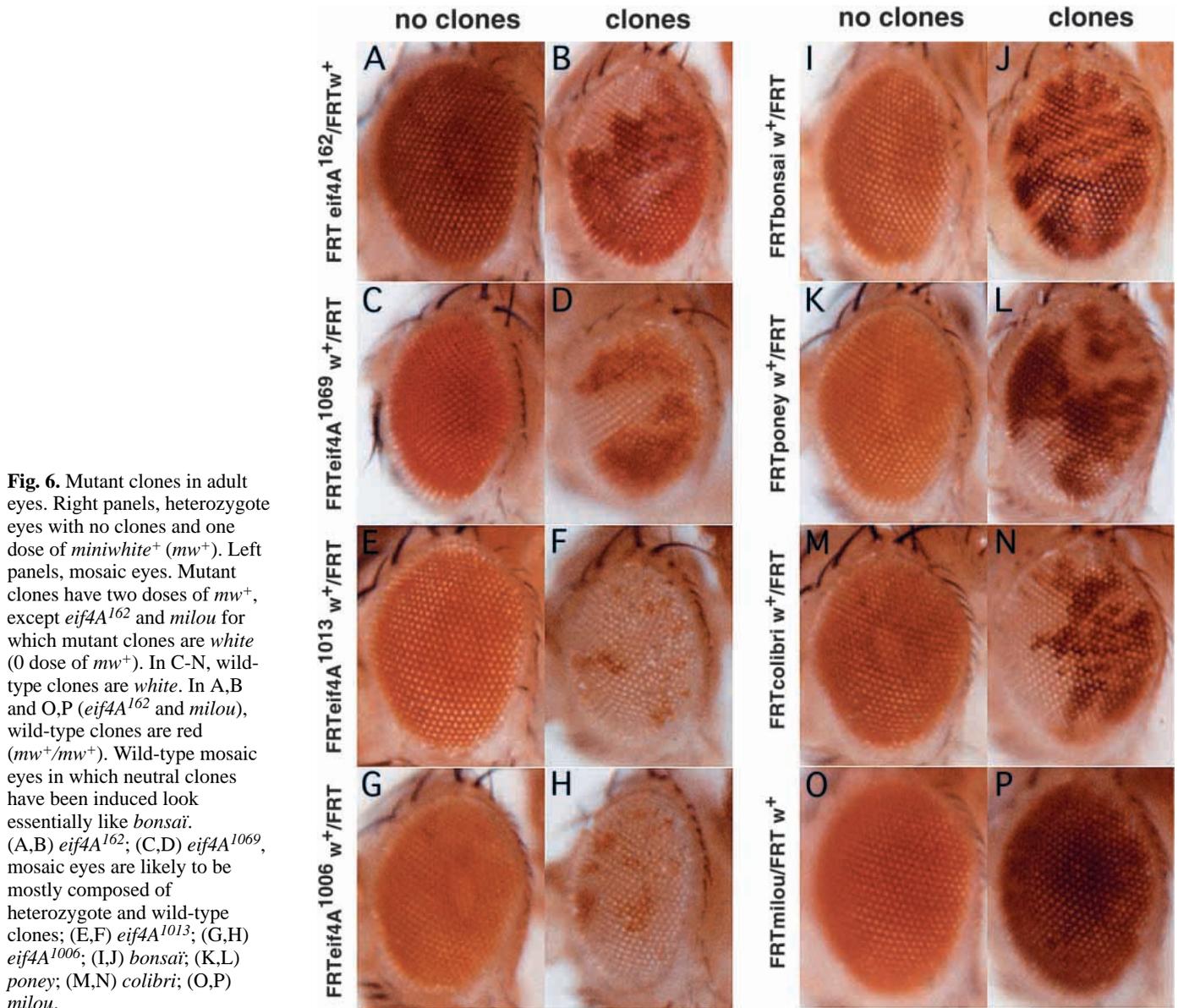
Fig. 6. Mutant clones in adult eyes. Right panels, heterozygote eyes with no clones and one dose of *miniwhite*[+] (*mw*[+]). Left panels, mosaic eyes. Mutant clones have two doses of *mw*[+], except *eif4A*[162] and *milou* for which mutant clones are *white* (0 dose of *mw*[+]). In C-N, wild-type clones are *white*. In A,B and O,P (*eif4A*[162] and *milou*), wild-type clones are red (*mw*[+]/*mw*[+]). Wild-type mosaic eyes in which neutral clones have been induced look essentially like *bonsaï*. (A,B) *eif4A*[162]; (C,D) *eif4A*[1069], mosaic eyes are likely to be mostly composed of heterozygote and wild-type clones; (E,F) *eif4A*[1013]; (G,H) *eif4A*[1006]; (I,J) *bonsaï*; (K,L) *poney*; (M,N) *colibri*; (O,P) *milou*.

different cell cycle/growth genes, raising the possibility that suboptimal levels of Eif4A and possibly other protein synthesis functions could be detrimental for translation of regulatory genes, but not for translation of their targets. This could potentially lead to an uncoupling between growth and DNA replication, and represent an important step toward the loss of growth and proliferation control.

Lack of production or activation, or downregulation of E2F/DP seems to occur in *eif4A*[1006] mutants. Does this reflect a general protein synthesis defect or a specific one? An attractive hypothesis is that the DNA replication block seen in *eif4A*[1006] mutants is due to a translation defect of a specific set of cell-cycle or growth regulatory products. It was previously suggested that certain genes might be more sensitive than others to Eif4A levels, i.e. homeotic genes (Dorn et al., 1993) and Notch (Röttgen et al., 1998). Our results suggest that genes involved in DNA replication initiation and/or exit from quiescence may also exhibit a specific sensitivity to *eif4A*-mediated translation. An E2F/DP regulator may be such an *eif4A* target. One likely candidate could be a positive regulator of E2F/DP activity, like the cyclinD/CDK4 kinase (for review, see Sherr, 1996). This is supported by the finding that expression of CLN3, the D-type cyclin's yeast counterpart, is regulated at the level of translation in response to growth signals (Polymenis and Schmit, 1997).

Another component of the Eif4F complex, the cap-binding protein Eif4E, has been proposed as the rate-limiting factor in translation initiation. *eif4E* mutants also show an LGD phenotype (T. P. Neufeld and B. A. Edgar, unpublished data). The allelic series of *eif4A* that we have characterized suggests that Eif4A may also be rate limiting in the translation of specific cell cycle or growth-related mRNAs, presumably containing complex UTRs.

milou, a mutant deficient in the exit from quiescence

milou mutants show a near absence of DNA replication

associated with a severe larval growth arrest and an extended larval period. DNA replication is almost normal in 1-day-old *milou* larvae, but shut down in 2-day-old mutants (not shown). This is similar to wild-type larvae starved from hatching, which synthesize DNA for a few hours before becoming quiescent (Britton and Edgar, 1998). Zygotic activation and/or maintenance of DNA replication does not seem to occur in this mutant. This could result from a mutation in a rate-limiting S-phase factor encoded by *milou*. Alternatively, *milou* could encode a gene required for growth. In this case, DNA replication could be downregulated as a consequence of the mutant's growth defect. We were able to bypass the DNA replication defect in *milou* by overexpressing E2F/DP. This suggests that this transcription factor fails to be made or activated in this mutant, and leads us to place *milou*'s function upstream of E2F/DP.

In contrast to the severe growth arrest observed in *milou* larvae, *milou* cells can proliferate in heterozygous hosts. *milou* clones also exhibit better growth than the other LGD mutants, apart from *bonsaï* (for example, compare in Fig. 4E,T, *milou*, with Fig. 4B,Q, *poney*, and Fig. 4D,S, *colibri*). However, adult wings in which *milou* clones have been induced frequently show large cuts (Fig. 5F) and *milou* mosaic eyes have a mildly roughened phenotype (Fig. 6H). The *milou* mosaic eye phenotype includes increased cell death, abnormally small ommatidia, loss of ommatidia rows and of interommatidial bristles (data not shown). This suggests that *milou* cells are unable to complete their proliferation program and then undergo apoptosis. *milou* larvae do not respond to nutritional signals to initiate the cell cycle and do not exit from organismal quiescence. Cycling *milou* cells may have retained their ability to proliferate in contrast to quiescent *milou* cells. The *milou* mutant could therefore exhibit a prominent proliferation defect at the exit from quiescence.

bonsaï, a mutant impaired in a non-cell-autonomous growth function

bonsaï mutant larvae exhibit a very strong growth retardation and never reach the normal size. A few of these mutants are able to metamorphose into small flies (20 days after hatching instead of 5) that die soon after eclosion. Based on the strong growth delay that is seen in the larvae and pupae, we expected to find a similar type of defect in *bonsaï* mutant clones. In fact, these clones were found to grow at a wild-type rate. This is in contrast to slow growing mutant clones induced in *eif4A*[162] for instance, which exhibit a less severe larval growth defect than *bonsaï* (compare Fig. 3B,Q and Fig. 4A,P), and to *Minute* mutations which also exhibit strong developmental delays (for review, see Lambertsson, 1998). *bonsaï* mosaic adults are normal, indicating that the mutant cells differentiate normally. The most-likely explanation for normal proliferation of *bonsaï* mutant cells is that these cells do not require *bonsaï* cell-autonomously and that their growth is rescued in a wild-type environment. This raises the possibility that Bonsaï could be involved in some aspects of growth signalling. *bonsaï* was found to encode a partial homolog of the prokaryotic ribosomal protein S15 and of the yeast mitochondrial ribosomal protein MRPS28 (Dang and Ellis, 1990; data not shown). This suggests that *bonsaï* encodes for a product involved in translation, potentially a mitochondrial ribosomal protein. How might such a protein synthesis-related gene be non-autonomous? Interestingly, ribosomal protein S6 has been implicated in regulated ecdysteroidogenesis in insect prothoracic glands (Song and Gilbert, 1994). Steroid hormone biosynthesis takes place in the cytoplasm but also in mitochondria (for review, see Zorov et al., 1997). Furthermore, certain tissues and cells are known to be enriched in mitochondria. Therefore, we propose that Bonsaï might be involved in tissue-specific growth factor production. *bonsaï* mutant brains appear to be underdeveloped as compared to the rest of the body (Fig. 5A,B). This may reflect a specific sensitivity of the brain to a growth-defective context. Alternatively, the brain could be the primary focus of the *bonsaï* mutation, the larval growth retardation being a consequence of this defect.

Organismal growth regulation pathways

The phenotypic analysis of different mutants found in our screens has allowed us to approach multiple aspects of organismal growth. Mutants such as *milou* and *eif4A*[1006] should help to understand how cells exit from quiescence in multicellular organisms. Mutants that do not grow and have an extended lifespan phenocopy the effects of starvation and are reminiscent of dauer larval formation in *Caenorhabditis elegans* (Riddle and Albert, 1997) or sporulation in yeast (Miller, 1989). Starvation may elicit similar survival responses in different species, and it is likely that homologous pathways are used to downregulate growth. It will be interesting to know if components of these pathways are responsible for nutrition/environment-related life mode adaptations such as diapause or hibernation. Finally, of particular interest is the characterization of non-autonomous growth defects such as *bonsaï* that should lead to identification of tissues and cells that can influence the growth of others. These mutants should help to describe the mechanisms underlying the coordination of growth of multiple organs and to better understand the contributions of autonomous and non-autonomous factors in the control of organ size (Bryant and Simpson, 1984). Translation regulation appears to be a major mechanism by which growth and cell cycle are controlled in response to growth signals. Selective translation of regulatory proteins rate-limiting for cell cycle or growth progression appears to be critical, particularly at the exit from quiescence. *eif4A*, *poney* and *colibri* mutant cells show different growth rates in eye and wing discs. Endoreplicative and diploid cell cycles also seem to have different requirements for Eif4A, since larval size and DNA replication levels are very different between *eif4A*[162] and *eif4A*[1069], but not mutant clone size. It is possible that these different growth modes reflect tissue-specific differences in protein synthesis requirement.

Several models can be proposed to explain the coordinated growth of several tissues within an organism. One model is that all tissues grow autonomously, according to nutrient availability. In a second model, all tissues respond to a growth signalling center. In a third model, tissues grow coordinately via a relay system: for example, tissue 1, which senses nutritional cues, signals to tissue 2, which signals to tissue 3, which signals to tissue 4. A relay model of this type could account for the greatest number of growth modulations and could accomodate numerous observations. For example, in humans, growth hormone is produced by the brain and acts on the liver to produce insulin-like growth factor 1 (IGF-1), and IGF-1 in turn

affects growth of the whole body (Heyner and Garside, 1994). Perhaps similarly, ecdysone is synthesized in the ring gland and activated in the fat body in flies (for review, see Riddiford, 1993), or the midgut in *Manduca sexta* (Mayer et al., 1978). Although much work still needs to be done to decrypt the pathways that regulate growth in multicellular organisms, we believe that the study of mutant collections, such as the one presented here will help to understand the basis of growth signalling and cell cycle regulation in whole organisms.

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* **215**, 403-410.

Bier, E., Vaessin, H., Shepherd, S., Lee, K., McCall, K., Barbel, S., Ackerman, L., Carretto, R., Uemura, T., Grell, E., Jan, L. Y. and Jan, Y. N. (1989). Searching for pattern and mutation in the *Drosophila* genome with P-lacZ vector. *Genes Dev.* **3**, 1273-1287.

Bodenstein, D. (1950). The post-embryonic development of *Drosophila*. In *Biology of Drosophila* (ed. A. P. Demerec), pp. 285-287. New York: John Wiley & Sons.

Britton, J. S., and Edgar, B. A. (1998). Environmental control of the cell cycle in *Drosophila*: nutrition activates mitotic and endoreplicative cells by distinct mechanisms. *Development* **125**, 2149-2158.

Bryant, P. J. and Simpson, P. (1984). Intrinsic and extrinsic control of growth in developing organs. *Q. Rev. Biol.* **59**, 387-415.

Church, R. B. (1965). Genetic and biochemical studies of growth in *Drosophila*. Doctoral Thesis. University of Edinburgh, Edinburgh.

Craig, A. W., Haghighat, A., Yu, A. T. and Sonenberg, N. (1998). Interaction of polyadenylate-binding protein with the eIF4G homologue PAIP enhances translation. *Nature* **392**, 520-523.

Dang, H. and Ellis, S. R. (1990). Structural and functional analyses of a yeast mitochondrial ribosomal protein homologous to ribosomal protein S15 of *Escherichia coli*. *Nucl. Acids Res.* **18**, 6895-6901.

Darwin, C. (1859). Ch. 5: Laws of variation. In *The Origins of Species*, First Edition.

Dorn, R., Morawietz, H., Reuter, G. and Saumweber, H. (1993). Identification of an essential Drosophila gene that is homologous to the translation initiation factor eIF-4A of yeast and mouse. *Mol. Gen. Genet.* **237**, 233-240.

Duronio, R. J., O'Farrell, P. H., Xie, J.-E., Brook, A. and Dyson, N. (1995). The transcription factor E2F is required for S phase during *Drosophila* embryogenesis. *Genes Dev.* **9**, 1445-1455.

Edgar, B. A. and Lehner, C. F. (1996). Developmental control of cell cycle regulators: a fly's perspective. *Science* **274**, 1646-1652.

Fain M. J. and Stevens B. (1982). Alterations in the cell cycle of *Drosophila* imaginal disc cells precede metamorphosis. *Dev. Biol.* **92**, 247-58.

Golic, K. G. and Lindquist, S. (1989). The FLP recombinase of yeast catalyzes site-specific recombination in the *Drosophila* genome. *Cell* **59**, 499-509.

Heyner, S. and Garside, W. T. (1994). Biological actions of IGFs in mammalian development. *BioEssays* **16**, 55-57.

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

Mathews, M. B., Sonenberg, N. and Hershey, J. W. B. (1996). Origins and targets of translational control. In *Translational Control* (ed. J. W. B. Hershey and M. B. Mathews), pp. 1-29. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Mayer, R. T., Svoboda, J. A. and Weirich, G. F. (1978). Ecdysone 20-hydroxylase in midgut mitochondria of Manduca sexta (L.). *Hoppe Seylers Z. Physiol. Chem.* **359**, 1247-1257.

Merrick, W. C. and Hershey, J. W. B. (1996). The pathway and mechanism of eukaryotic protein synthesis. In *Translational Control* (ed. J. W. B. Hershey and M. B. Mathews), pp. 31-69. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Migeon, J. C., Garfinkel, M. S. and Edgar, B. A. (1999). Cloning and characterization of *peter pan*, a novel *Drosophila* gene required for larval growth. *Mol. Biol.Cell.* (in press)

Miller, J. J. (1989). Sporulation in *Saccharomyces cerevisiae*. In *The Yeasts* vol.3 Second Edition (ed. A. H. Rose and J. S. Harrison), pp. 491-550. New York: Academic Press.

Morata, G. and Ripoll, P. (1975). Minutes: mutants of *Drosophila* autonomously affecting cell division rate. *Dev. Biol.* **42**, 211-221.

Pellicena-Palle, A. and Salz, H. K. (1995). The putative *Drosophila* NMDARA1 gene is located on the second chromosome and is ubiquitously expressed in embryogenesis. *Biochim. Biophys. Acta* **1261**, 301-303.

Pirotta, V. (1986). Cloning *Drosophila* genes. In *Drosophila: A Practical Approach* (ed. D. B. Roberts), pp. 83-110. Oxford: IRL Press.

Polymenis, M. and Schmidt, E. V. (1997). Coupling of cell division to cell growth by translational control of the G_1 cyclin CLN3 in yeast. *Genes Dev.* **11**, 2522-2531.

Qu, S., Perlaky, S. E., Organ, E. L., Crawford, D. and Cavener, D. R. (1997). Mutations at the Ser50 residue of translation factor eIF-2alpha dominantly affect developmental rate, body weight, and viability of *Drosophila melanogaster*. *Gene Express.* **6**, 349-360.

Quiring, R., Walldorf, U., Kloter, U. and Gehring, W. J. (1994). Homology of the *eyeless* gene of *Drosophila* to the *Small eye* gene in mice and *Aniridia* in humans. *Science* **265**, 785-789.

Riddiford, L. M. (1993). Hormones and *Drosophila* development. In *The Development of Drosophila melanogaster II*. (ed. M. Bate and A. Martinez-Arias), pp. 899-939. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Riddle, D. L. and Albert, P. S. (1997). Genetic and environmental regulation of dauer larva development. In *C. elegans II*. (ed. D. Riddle, B. Meyer, J. Priess and T. Blumenthal), pp. 739-768. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Röttgen, G., Wagner, T. and Hinz, U. (1998). A genetic screen for elements of the network that regulates neurogenesis in *Drosophila*. *Mol. Gen. Genet.* **257**, 442-451.

Royzman, I., Whittaker, A. J. and Orr-Weaver, T. L. (1997). Mutations in *Drosophila* DP and E2F distinguish G1-S progression from an associated transcriptional program. *Genes Dev.* **11**, 1999-2011.

Sherr, C. J. (1996). Cancer cell cycles. *Science* **274**, 1672-1677.

Simpson, P. (1979). Parameters of cell competition in the compartments of the wing disc of *Drosophila*. *Dev. Biol.* **69**, 182-193.

Smith, A. V. and Orr-Weaver, T. L. (1991). The regulation of the cell cycle during *Drosophila* embryogenesis – the transition to polyteny. *Development* **112**, 997-1008.

Song, Q. and Gilbert, L. I. (1994). S6 phosphorylation results from prothoracicotropic hormone stimulation of insect prothoracic glands: a role for S6 kinase. *Dev. Genet.* **15**, 332-338.

Talamillo, A., Chisholm, A. A. K., Garesse, R. and Jacobs, H. T. (1998). Expression of the nuclear gene encoding mitochondrial ATP synthase subunit alpha in early development of Drosophila and sea urchin. *Mol. Biol. Rep.* **25**, 87-94.

The, I., Hannigan, G. E., Cowley, G. S., Reginald, S., Zhong, Y., Gusella, J. F., Hariharan, I. K. and Bernards, A. (1997). Rescue of a Drosophila NF1 mutant phenotype by protein kinase A. *Science* **276**, 791-794.

Török, T., Tick, G., Alvarado, M. and Kiss, I. (1993). *P-lacW* insertional mutagenesis on the second chromosome of *Drosophila melanogaster*: Isolation of lethals with different overgrowth phenotypes. *Genetics* **135**, 71-80.

Xu, T. and Rubin, G. M. (1993). Analysis of genetic mosaics in developing and adult *Drosophila* tissues. *Development* **117**, 1223-1237.

Zaffran, S., Chartier, A., Gallant, P., Astier, M., Arquier, N., Doherty, D., Gratecos, D. and Semeriva, M. (1998). A *Drosophila* RNA helicase gene, *pitchoune*, is required for cell growth and proliferation and is a potential target of d-Myc. *Development* **125**, 3571-3584.

Zorov, D. B., Krasnikov, B. F., Kuzminova, A. E., Vysokikh, M. Y. and Zorova, L. D. (1997). Mitochondria revisited. Alternative functions of mitochondria. *Biosci. Rep.* **17**, 507-520.