Regena (*Rga*), a Drosophila Homolog of the Global Negative Transcriptional Regulator *CDC36* (*NOT2*) from Yeast, Modifies Gene Expression and Suppresses Position Effect Variegation

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ABSTRACT

A mutation in *Regena* (*Rga*) was isolated in screens for modifiers of *white* eye color gene expression. The reduction in the level of the *Rga* product results in a complex modulation of *white* mRNA both positively and negatively, depending on the developmental stage. In addition to *white*, *Rga* also affects the expression of several other tested genes, with one of them, *Vinculin*, being regulated in a strong sex-specific manner. *Rga* was cloned by transposon tagging. Its predicted product lacks any recognized nucleic acid–binding motif but is homologous to a global negative transcriptional regulator, *CDC36* (*NOT2*), from yeast. *Rga* also acts as a suppressor of position effect variegation, suggesting that a possible function of *Rga* could be mediation of an interaction between chromatin proteins and the transcriptional complex.

TRANSCRIPTIONAL initiation is controlled by the presence and activity of specific nuclear factors that directly bind to the short sequence motifs in the promoter regions of genes, thus, interacting with components of the general transcription machinery. Regulation can also be affected by intermediary proteins that govern transcription indirectly by association with the promoter via protein-protein interactions. Their function is to mediate the interaction of transcription factors with the RNA polymerase II complex or to establish or maintain the proper chromatin structure. For a particular gene, a unique combination of all available regulatory proteins and *cis*-acting elements determines its specific expression pattern in development.

The thorough studies of intermediary proteins in yeast resulted in identification and molecular characterization of a set of global positive and negative regulators affecting the expression of numerous genes (for review, see Struhl 1993). Based on genetic and biochemical analyses, it was proposed that some of them act as heterologous multiprotein complexes. For example, Cdc39p (Not1p), Cdc36p (Not2p), Not3p, and Mot2p (Not4p) presumably form a 500-kD complex that inhibits transcription by affecting the ability of transcription factor TFIID for TATA element utilization (Collart and Struhl 1994). Another potential complex is formed by Spt4p, Spt5p, and Spt6p, which negatively regulate transcription of several loci. Unlike Not proteins, these were hypothesized to repress gene expression by affecting chromatin structure (Swanson and Winston 1992) analogous to that postulated for Polycomb group proteins (Paro 1990). One such component, Spt6p, controls chromatin structure by direct interactions with histones H3 and H4 (Bortvin and Winston 1996).

The *white* locus provides a useful model system in Drosophila where such interactions can be studied. A large number of genes that modify *white* expression have been identified (Bingham and Zachar 1985; Chapman and Bingham 1985; Rabinow *et al.* 1991; Birchler *et al.* 1994; Bhadra and Birchler 1996; Bhadra *et al.* 1997a,b). Because such modifiers usually produce a dosage effect, they were proposed to be examples of genes that are the underlying basis of aneuploid syndromes (Birchler and Newton 1981) and various types of dosage compensation (Devlin *et al.* 1982; Birchler *et al.* 1989, 1990; Birchler 1996; Bhadra *et al.* 1997a,b). The *zeste* gene, the only previously cloned modifier of *white*, encodes a protein that specifically binds to transcriptional enhancers in the vicinity of the promoter region of the *white* gene and controls long-distance interaction in chromosomes (Benson and Pirrotta 1987; for review, see Pirrotta 1990). The *zeste* product shows extensive self-assembly properties and can form multimeric complexes connecting distantly located regions of DNA to one another (Bickel and Pirrotta 1990). Several other modifiers have been found to interact with the *white* gene. They produce both direct correlative and inverse dosage effects on *white* expression. For example, mutant alleles of the *Weakener-of-white* (*Wow*) locus increase the level of the *white* mRNA in larvae but decrease it in adults (Birchler *et al.* 1994). *Modifier of white* (*Mow*) was shown to have a differential effect on gene expression in males and fe-

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males (Bhadra and Birchler 1996). *Inverse regulator-a* (*Inr-a*) modifies the *white* gene only in larval and pupal stages, when the majority of pigment is deposited (Rabinow *et al.* 1991). In addition to *white*, two other tested genes, *brown* and *scarlet*, are also affected by these modifiers. Moreover, *Wow* and *Mow* were recognized as weak suppressors of position effect variegation (PEV; Birchler *et al.* 1994; Bhadra and Birchler 1996), which implies a connection between regulation of higher order chromatin structure and modulation of gene activity.

Using *white*, we are interested in isolating as complete a set of regulators as possible for a single target locus to understand how the numerous modifiers affect gene expression. In this report, a newly identified Drosophila gene, *Regena* (*Rga*), which modulates the expression of the *white* locus, is characterized. The product of *Rga* is essential for normal Drosophila viability, as evidenced by the fact that the mutant allele induced by a *P*-element insertion is semilethal. *Rga* was found to act as a suppressor of PEV and regulate the expression of several unrelated genes. Because it affects expression of numerous genes, it was named *Regena* (*Rga*) (*Regulator of gene activity*). The predicted protein product contains a domain homologous to the yeast general negative transcription regulator *CDC36* (*NOT2*).

MATERIALS AND METHODS

Fly stocks: Flies were raised on standard Drosophila media at 25°. Genetic markers used here can be found in Lindsley and Zimm (1992).

l(3)03834, a single *PZ* element (Mlodzik and Hiromi 1992) insertion on chromosome 3 (83B4-5), was identified in a large screen for autosomal mutations affecting the eye color of *white-apricot* flies. To determine whether this insertion is responsible for the mutant phenotype, the *P* element was mobilized by crossing *l(3)03834/TM3, Sb* flies to the *delta 2-3, Sb/TM6, Ubx* strain (Robertson *et al.* 1988). The F_1 *l(3)03834/delta 2-3, Sb* males were crossed to the balancer stock *TM3, Ser/MKRS* individually. The *Sb*, non-*Ser* progeny (*l(3)03834/MKRS*) were screened for *rosy*$^-$ flies, which were mated to *TM3, Ser/MKRS* to establish a stock.

For the developmental Northern analysis, genetic crosses were performed essentially as described (Birchler *et al.* 1994). Briefly, *l(3)03834/TM3, Sb* females were crossed to *T(2;3)CyO, Cy Tb ch* translocation males. The F_1 males containing this translocation heterozygous with *l(3)03834* were mated to Canton S females. The *Tb* marker allows discrimination between +/+ and *l(3)03834*/+ classes at the larval and pupal stages, while the *Curly* marker allows this distinction in adults.

DNA manipulation and cDNA libraries: All standard DNA manipulations were performed as described in Sambrook *et al.* (1989).

Genomic DNA was isolated from 50 flies by standard procedures with some modifications (Alatortsev 1988). The fragment flanking the insertion site in *l(3)03834* was rescued as described in Karpen and Spradling (1992). The enzymes *Xba*I and *Nhe*I were used for double digestion of 1 μg of the genomic DNA isolated from flies carrying the *P* element insertion. After inactivation and dilution, a ligation reaction was performed in 200 μl at 16° overnight. The mixture was precipitated and used for electroporation of *Escherichia coli DH5*α.

The P1 phage 81-95, containing wild-type DNA from the 83B3-6 region on the cytological map (FlyBase http://flybase.bio.indiana.edu:82), was used to obtain an overlapping set of the fragments for cDNA library screens.

Two cDNA libraries were screened. One was prepared from eye imaginal disks of a wild-type stock and cloned into the λEX*lox* vector (Novagen, Madison, WI). The second was prepared from 2-wk-old male and female wild-type adults (Canton S) in the λZAP II vector (Stratagene, La Jolla, CA). Both libraries were screened by standard protocols (Sambrook *et al.* 1989). Eventually, 80,000 phage from the eye imaginal disk cDNA library and 1,200,000 phage from the adult cDNA library have been screened.

For sequencing, DNA fragments were cloned into the pSP72 (Promega, Madison, WI) or Bluescript II SK (Stratagene) vector. To obtain nested clones for sequencing, a gamma-delta transposon-based system was used (Strathmann *et al.* 1991). Sequencing was performed on a Sequi-Gen GT nucleic sequencing cell (Bio-Rad, Richmond, CA) using the Sequenase (v.2.0) kit (Amersham, Arlington Heights, IL).

Sequence analysis and multiple alignments were performed using DNA STAR software (DNASTAR, Madison, WI). Homology searches were performed at the National Center for Biotechnology Information's BLAST WWW Server.

Isolation and analysis of RNA: RNA was prepared according to the method of Chomczynski and Sacchi (1987). Flies were homogenized in a solution of 4 m guanidinium thiocyanate, 25 mm sodium citrate (pH 7.0), 0.5% sarcosyl, and 0.1 m 2-mercaptoethanol. A 0.1-volume of 2 m sodium acetate (pH 4.0), an equal volume of phenol, and a 0.2-volume of chloroform were added, sequentially. After centrifugation for 5 min and precipitation with isopropanol, the RNA was dissolved in 100 μl of homogenization solution and precipitated with an equal volume of isopropanol. The pellet was collected by centrifugation, washed with 70% ethanol, and stored at −70°. For Northern analysis, RNA samples were prepared in running buffer (20 mm morpholino propane sulfonic acid, 5 mm sodium acetate, and 1 mm EDTA) with 2.2 m formaldehyde and 50% (v/v) deionized formamide, and were then heat denatured for 5 min at 65°. The RNA was fractionated in running buffer on 1% agarose gels containing 2.2 m formaldehyde. The RNA was transferred by capillary blotting in 20× SSPE from the gel to neutral nylon membranes (Qiagen, Chatsworth, CA) and was then hybridized to radiolabeled antisense RNA probes prepared as described before (Birchler *et al.* 1990). The prehybridization and hybridization solutions were 0.5 m NaCl, 10% dextran sulfate, 0.2 mg/ml heparin, 1% sarkosyl, 0.1 m sodium phosphate (pH 7.0), 0.1% ficoll, 0.1% polyvinylpyrrolidone, 0.1% sodium pyrophosphate, and 50% (v/v) formamide. The filters were washed three times in a solution containing 5 mm sodium phosphate, 0.2% SDS, and 1 mm EDTA for 1 hr at 75°.

Antisense RNA probes were synthesized with T3, T7, or SP6 RNA polymerase (Promega). Description of the probes used here can be found in Birchler *et al.* (1990). The probe for the *Vinculin* gene is a cDNA cloned into the *Eco*RI site of the Bluescript II KS vector (Alatortsev *et al.* 1997).

RACE: To obtain 5′ and 3′ ends for *Rga*, the RACE protocol was performed using the Marathon cDNA Amplification Kit (Clontech, Palo Alto, CA) according to the manufacturer's recommendations. As a template for cDNA synthesis, the poly(A)$^+$ RNA was isolated from Canton S adult flies using an oligo(dT) cellulose (Sambrook *et al.* 1989). For PCR, the following primers were used: KK-1, AGCTGTTGGTTAGCT GTGGGT GTC; and M3, CCCACAGCTAACCAACAGCT. The RACE products were directly cloned into pGEM-T vector (Promega).

RESULTS

Isolation and characterization of the *P*-element insertion *l(3)03834*: To isolate autosomal genes involved in *trans*-regulation of *white* gene expression, the *white-apricot* allele was used to screen a set of lethal *P-lacZ* element insertions (Karpen and Spradling 1992) available from the Indiana University Drosophila Stock Center. The *apricot* allele produces a leaky, hypomorphic phenotype that allows detection of a wide range of modulation of *white* expression. The molecular basis of the lesion is a parallel insertion of the retrotransposon, *copia*, into the second intron of *white* (Gehring and Paro 1980; Bingham and Judd 1981; O'Hare *et al.* 1984). Among several isolated *P*-element insertions exhibiting both elevated and decreased pigment intensity on the *white-apricot* background, *l(3)03834* has been chosen for further study. This insertion maps to chromosome *3* at 83B4-5 and yields a darkened w^a phenotype.

To determine the phase of lethality, *l(3)03834/TM3* flies were crossed to the wild-type stock Canton S and the progeny were mated together. The number of embryos, larvae, pupae, and adults were scored. As many as 89% of the embryos hatched, compared with 94% for Canton S; however, only 72% of third instar larvae were observed. This number was the same for pupae and adults. Thus, the lethality of *l(3)03834* occurs at the embryonic and early larval developmental stages.

To confirm that the darker eye color and lethality attributed to *l(3)03834* are caused by the same insertion, the *P* element was mobilized by supplying transposase with the Δ2-3 chromosome (Robertson *et al.* 1988). Genomic DNA isolated from 116 independent $rosy^-$ stocks was subjected to Southern and PCR-based analyses. About 25% of the established stocks contain internal deletions within the *P* element, but no external deletions encompassing sequences outside of the *P* element were found. The other 75% appear to be precise excisions at this level of analysis. Sequencing of the region containing the insertion site (see below) from one of those, $l(3)03834^{rev}$, shows that this reversion is accompanied by loss of the element and one copy of the 8-bp duplication, thus restoring the wild-type sequence at the insertion site. The chromosome carrying $l(3)03834^{rev}$ was shown to be viable as a homozygote and shown to give a reversion of the eye color phenotype.

To characterize further and determine the nature of the effect of *l(3)03834* on the *white* gene, the interaction of *l(3)03834* with different *white* alleles, such as point mutations, transposable element insertions, lesions in the structural and regulatory regions, as well as an *Adh* promoter-*white* fusion construct, was examined. These tests were performed by crossing females carrying different *white* alleles to *l(3)03834/TM3, Sb* males. The eye color of the *l(3)03834/+* males was compared to that of their *TM3/+* brothers at the same age. If *l(3)03834* affects *white* transcription, one would expect that some alleles altering the 5′ region of *white* would not respond. On the other hand, if *l(3)03834* is involved in pigment deposition, then the *white* alleles would not alter the effect and all such flies would show a difference. The results of these tests are summarized in Table 1.

In brief, *l(3)03834* elevates the expression of the *white* gene in a subset of point and insertional mutations. Some are insertions of the retroelements, *copia* (w^a; Bingham and Judd 1981), *blood* (w^{bl}; Bingham and Chapman 1986), and *coral* (w^{co}; A. Csink, unpublished results) in the second or fifth intron of the *white* gene. Another affected allele, w^{IR1}, results from the insertion of the retroposon *I* element in the fifth intron (Sang *et al.* 1984; Fawcett *et al.* 1986). The same effect was found for most of the point mutations, which would imply that *l(3)03834* acts by changing the amount of transcripts from the wild-type *white* promoter. Indeed, no interaction has been found with the w^{sp2} allele, which is a deficiency in the 5′ regulatory region (Zachar and Bingham 1982). Another set of regulatory lesions unaffected by *l(3)03834* are reversions of the original $white^1$ mutation. This mutation is caused by the insertion of a *Doc* element in the 5′ leader of the *white* transcript (Driver *et al.* 1989). Three tested reversions, w^{eosin}, $w^{apricot\text{-}like}$, and w^{honey} were shown to be secondary insertions in the *Doc* retroposon (O'Hare *et al.* 1991). The former two do not exhibit dosage compensation between males and females. Another nondosage compensating allele, w^{ivory}, is also not affected. Finally, no response was found for the *Adh* promoter-*white* structural gene construct (Birchler *et al.* 1990). Thus, the allele specificity tests reveal the necessity of the proper *white* regulatory sequences for interaction with *l(3)03834*. Indeed when the $zeste^1$ mutation represses *white* expression, a response to *l(3)03834* no longer occurs.

In addition to the above-mentioned *white* alleles, a rearrangement chromosome, $In(1)w^{m4h}$, was also tested. This inversion contains the *white* gene juxtaposed to the centromeric heterochromatin of the *X* chromosome, which causes *white* inactivation in a mosaic manner (Reuter and Wolff 1981), referred to as PEV (Spofford 1976; Reuter and Spierer 1992; Henikoff 1996). *l(3)03834* was found to suppress the mottled phenotype of the *white* gene in $In(1)w^{m4h}$. This suppression is not as strong as in the case of previously described suppressors [*Su(var)s*], which as heterozygotes usually give practically wild-type eye color (Reuter *et al.* 1990), but it is rather comparable in its strength with three other *white* modifiers, *Wow*, *Lip*, and *Mow*, which are also suppressors of PEV (Birchler *et al.* 1994; Csink *et al.* 1994; Bhadra and Birchler 1996). To quantitate the effect of *l(3)03834* on PEV, pigment assays were performed on the segregating populations (Table 2). Spectrophotometric measurement demon-

TABLE 1

Effect of *l(3)03834* on selected alleles of the *white* gene

Allele	Interaction	*white* Locus lesion	Reference
w^a (apricot)	+	*copia* retrotransposon insertion in intron 2	Gehring and Paro (1980); Bingham and Judd (1981)
w^{IR1}	+	*I*-element insertion	Sang *et al.* (1984)
w^{bl} (blood)	+	*blood* retrotransposon in intron 2	Zachar and Bingham (1982); Bingham and Chapman (1986)
w^{co} (coral)	+	*coral* retrotransposon in intron 5	A. Csink, unpublished data
w^i (ivory)	None	Duplication of sequences from intron 1 to start of exon 3	Karess and Rubin (1982); Collins and Rubin (1983); O'Hare *et al.* (1984)
w^{sp} (spotted)	+	*B104* retrotransposon in 5′ regulatory region	O'Hare *et al.* (1984); O'Hare *et al.* (1983); Zachar and Bingham (1982)
w^{sp2} (spotted-2)	None	Deficiency in 5′ regulatory region	Zachar and Bingham (1982)
w^{sp3} (spotted-3)	+	Deficiency in 5′ regulatory region	Davison *et al.* (1985); Zazhar and Bingham (1982)
w^{sp55} (spotted-55)	+	*mdg3* retrotransposon in 5′ untranslated leader	Zachar and Bingham (1982); A. Csink, unpublished data
w^{sp81d5} (spotted-81d5)	+	Deficiency in 5′ regulatory region	Davison *et al.* (1985)
w^{a2} (apricot-2)	None/−	Point	Zachar and Bingham (1982)
w^{a3} (apricot-3)	None	Point	Zachar and Bingham (1982)
w^{col} (colored)	None	Point	Zachar and Bingham (1982)
w^{crr} (carrot)	+	Point	Zachar and Bingham (1982)
w^{Bwx} (Brownex)	+	Point	Zachar and Bingham (1982)
w^{cf} (coffee)	+	Point	Zachar and Bingham (1982)
w^h (honey)	None	*B104* element into *Doc* element of w^1	O'Hare *et al.* (1991)
w^e (eosin)	None	Transposable element reversion of w^1 (*Doc* element)	Hazelrigg (1987); O'Hare *et al.* (1984); Zachar and Bingham (1982)
w^{apl} (apricot-like)	None	P-M hybrid dysgenic revertant of w^1 (*Doc* element)	C. McElwain (personal communication)
z DP(1;1)w^{+61e19}	None	Duplication of *white* locus sequences	Gunaratne *et al.* (1986)
Adh-w #2	None	*Adh* promoter-*white* structural gene on chromsome *3*	Birchler *et al.* (1990)

In this table, − denotes enhancement and + denotes suppression. Females carrying the various *X*-linked *white* alleles were crossed to males carrying *l(3)03834* mutation over *TM3* balancer chromosome marked by *Sb*. *l(3)03834*/+ males were then compared to their *TM3*/+ brothers.

strates that *l(3)03834* significantly elevates pigment level in $In(1)w^{m4h}$; *l(3)03834*/+ males relative to their $In(1)w^{m4h}$; *TM3*/+ brothers. To exclude the possibility that the observed effect is caused by the presence of a preexisting hypothetical mutation that suppresses PEV other than the *P*-element insertion, the same analysis was done for revertant flies. The data presented in Table 2 show that the amount of pigment in $In(1)w^{m4h}$; $l(3)03834^{rev}$/+ and $In(1)w^{m4h}$; *TM3*/+ is similar.

To test whether the suppressing effect of *l(3)03834* on the $In(1)w^{m4h}$ chromosome is caused by a general suppression of PEV rather than a specific interaction with the *white* gene, the effect of *l(3)03834* on a variegating allele of *yellow* in the inversion $In(1)y^{3P}$ was examined. For segregating classes, *l(3)03834*/*TM3* males were crossed to $In(1)y^{3P}$ females, and the F_1 males segre-

TABLE 2

Pigment assay results of *l(3)03834* on $In(1)w^{m4h}$ in males

Genotype	OD_{480}	Ratio
w^{m4h}; *l(3)03834*/+	0.269 ± 0.032*	3.41
w^{m4h}; *TM3*/+	0.079 ± 0.006	
w^{m4h}; $l(3)03834^{rev}$/+	0.063 ± 0.003	1.13
w^{m4h}; *TM3*/+	0.056 ± 0.003	

The effect of *l(3)03834* on $In(1)w^{m4h}$ is determined from the OD_{480} values, which were measured in eye pigment assays on males of each genotype in triplicate. As a control, the same values were calculated for the $l(3)03834^{rev}$ flies. The value marked with an asterisk is significantly different from the control at the 95% confidence level in a *t*-test.

gating for *l(3)03834* and the *TM3* balancer were scored. The number of wild-type and *yellow* triple row bristles along the anterior margin of the wing blades were counted in each class of flies (Table 3). *l(3)03834* reduces the frequency of *yellow* variegation among the *In(1)y^{3P}* flies thereby suppressing the *yellow* bristle variegation nearly threefold (3.3%) above the comparable control values (10.9%). On the contrary, the revertant flies show no suppression of *yellow* variegation. Thus, *l(3)03834*, but not the revertant, suppresses variegation of both *white* and *yellow* in two different rearrangements, implying that *l(3)03834* is a general modifer of PEV.

Effect of *l(3)0384* on the transcripts of unrelated genes: Because *l(3)03834* was implicated in the interaction with the regulatory sequences of the *white* gene, its effect on *white* transcripts was studied. To determine how general this effect is, the steady-state mRNA level of two related genes, *brown* (*bw*; Dreesen *et al.* 1988) and *scarlet* (*st*; Tearle *et al.* 1989), together with four unrelated genes, *rudimentary* (*r*; Segraves *et al.* 1984), *Phosphogluconate dehydrogenase* (*Pgd*; Scott and Lucchesi 1991), *Glycerol phosphate dehydrogenase* (*Gpdh*; von Kalm *et al.* 1989), and *Vinculin* (*Vinc*; Alatortsev *et al.* 1997) were examined. RNA isolated from two different populations of adults segregating for the *l(3)03834* and balancer chromosomes was subjected to Northern analysis. As a control for normal expression, the same analysis was performed for the revertant flies *l(3)03834^{rev}*. Triplicate RNA transfers were hybridized with probes for the genes mentioned above. A probe for the *rp49* gene (Kongsuwan *et al.* 1985) was used as a gel-loading control because, in the case of the mutation, there are no differences found for the level of *rp49* transcripts when compared to rRNA (data not shown). The results of the phosphorimagery scans are given in Table 4. Briefly, the level of *bw*, *st*, and *r* expression is not significantly affected by *l(3)03834*. The other tested genes are affected but the level of their expression is usually greater in males than in females. For example, the transcripts of *white* in males are increased, while in females they are decreased. The level of *Pgd* and *Gpdh* mRNAs were found to decrease in both males and females. However, the most dramatic effect was observed for the *Vinc* gene. Although it is not affected in males, the transcripts in females are strongly decreased, which results in a very low amount of product. We considered the possibility that the decreased steady-state level of *Vinculin* mRNA is caused by an effect on the tissue or cells in which *Vinculin* is normally expressed. However, examination of mutant larvae and adult flies did not reveal any developmental changes of primordia or adult structures, particularly ovaries, testis, imaginal discs, and brain. Vinculin was shown to play an important role in processes of cell motility and adhesion as one of the main counterparts of linkages between actin filaments and transmembrane receptors (Kemler 1993). The normal viability observed in flies with an extremely low expression of the *Vinc* gene confirms the previous results of the nonessentiality of Vinculin in Drosophila (Alatortsev *et al.* 1997). Thus, *l(3)03834* exerts an effect on the steady-state RNA level, and this effect is not restricted to the *white* gene. The observation that four of seven tested genes respond to *l(3)03834* significantly implies that *l(3)03834* could be involved in general regulation of gene expression.

To study the effect of the *l(3)03834* mutation on the *white* mRNA level in larvae and pupae, crosses were performed as described in materials and methods. Developmentally staged samples were collected from third instar larvae, early, mid-, and late pupae. Triplicate RNA transfers were hybridized with *white* and *rp49*

TABLE 3

The effect of *l(3)03834* on *yellow* variegation in *In(1)y^{3P}*

Genotype	Total number of bristles	Frequency of triple row bristles: percent *yellow$^+$*	Frequency of triple row bristles: percent *yellow*
y^{3P}; *l(3)03834* / +	1873	96.7	3.3*
y^{3P}; *TM3* / +	1782	89.1	10.9
y^{3P}; *l(3)03834^{rev}* / +	1895	87.5	12.5
y^{3P}; *TM3* / +	1939	86.9	13.1

The value marked with an asterisk is significantly different from the control at the 95% confidence level.

TABLE 4

Quantitation of the effect of *l(3)03834* on gene expression in adults

Gene	Male	Female
*white***	1.15 ± 0.02*	0.64 ± 0.02*
brown	1.12 ± 0.05	0.81 ± 0.06
scarlet	0.89 ± 0.06	0.88 ± 0.13
rudimentary	1.37 ± 0.19	1.03 ± 0.02
Pgd	0.77 ± 0.03*	0.64 ± 0.06*
Gpdh	0.83 ± 0.04*	0.73 ± 0.03*
*Vinculin***	1.13 ± 0.06	0.37 ± 0.04*

Northern blots for each studied gene were performed in triplicate. The blots were quantitated after hybridization using a phosphorimager (Fuji Medical Systems USA/Bio Images Bioimaging Group, Stamford, CT). The values obtained for each lane were divided by that of *rp49*, which was used as a control. The value for the *l(3)03834* / + class was divided by the + / + class and, in parallel, the value for *l(3)03834^{rev}* / + was divided by the + / + class. Finally, the ratio obtained for the *l(3)03834* class was normalized to that obtained for *l(3)03834^{rev}*. These ratios, obtained for each blot, were used for calculation of the mean and the standard errors. Those ratios marked with a single asterisk are significantly different from 1 at the 95% confidence level in a *t*-test. Genes marked with a double asterisk have the level of expression in females significantly different from that in males at the 95% confidence level in a *t*-test.

TABLE 5

Quantitation of *white* transcripts from a segregating *l(3)03834* population

	Male	Female
Larvae	1.61 ± 0.05*	1.57 ± 0.05*
Early pupae	0.76 ± 0.02*	0.86 ± 0.03
Mid-pupae	1.10 ± 0.03	1.11 ± 0.02*
Late pupae	1.02 ± 0.02	1.14 ± 0.05

Northern blots for each developmental stage were performed in triplicate. The blots were quantitated using a Fuji phosphorimager. The values obtained for each lane were divided by that of *rp49*, which was used as a control. The value for the *l(3)03834*/+ class was divided by the +/+ class. These ratios were used for calculation of the mean and standard errors for the three replicas. Those ratios marked with an asterisk have the *l(3)03834*/+ class significantly different from the +/+ class at the 95% confidence level in a *t*-test.

antisense probes, the latter being used as a gel-loading control. The level of *rp49* does not vary relative to ribosomal RNA (data not shown). The results of phosphorimagery analysis are shown in Table 5. There is an elevation of *white* transcripts in larvae in *l(3)03834*/+ compared to +/+ individuals. In middle pupae, the effect returns to control levels. The phenotypic elevation of *white* expression could be caused by a short pulse of increase, similar to the larval effect, that occurs between our sampled stages—most likely at the late pupal/early adult transition preceding the adult stage, where a slight but significant increase was found (Table 6).

Cloning of the *Rga* gene and analysis of cDNA clones: To identify the gene responsible for the mutant phenotype, the genomic sequence flanking the *P*-element insertion was cloned via the plasmid rescue method. Screening a random and oligo(dT)-primed adult cDNA library yielded seven different clones falling into three regions, with the *P* element being inserted into the region of the 0.6-kb cDNA, c9.1 (Figure 1). A fragment of c9.1 revealed a single 2.8-kb mRNA on a Northern blot of wild-type RNA (Figure 2A). This mRNA will be subsequently referred to as the *Rga* mRNA. To study its transcription in the case of the mutation, a Northern blot analysis of total RNA from a segregating population of *l(3)03834* and that of *l(3)03834*^{rev} was performed. In the case of the mutation, the amount of the *Rga* mRNA is decreased to one-half relative to the respective *rp49* control in both males and females, and an aberrant 4.5-kb transcript appears (Figure 2D, lanes 3–4 and 9–10). The aberrant mRNA could result from termination of transcription within the *P* element, particularly within the *hsp-70* tail (Mlodzik and Hiromi 1992), which would give the transcript of the observed size. The transcript in the revertant *l(3)03834*^{rev} is restored to normal (Figure 2D, lanes 1 and 2 and 7 and 8). To study the transcription in mutant homozygotes, RNA was isolated from rare surviving flies, which do not carry the *TM3* balancer marker *Sb* and, hence, are homozygous for *l(3)03834*, as well as from heterozygotes *l(3)03834*/*TM3*. The 2.8-kb *Rga* mRNA is not detectable in mutant homozygotes (Figure 2D, lanes 5–6 and 11–12), and the steady-state level of *white* mRNA is increased in mutant homozygous males (Figure 2D and Table 6). Sequence analysis confirmed that the insertion of a *P* element occurs within c9.1 (see below). Thus, the 2.8-kb mRNA appears to correspond to the gene responsible for the mutant phenotype because the insertion of a *P* element causes a twofold reduction of this mRNA in the heterozygotes and an absence in homozygotes while in the revertant the RNA returns to normal.

The cDNA c9.1 is only 0.6 kb in length, implying that it is an incomplete copy. Because we failed to isolate a longer cDNA, a rapid amplification of cDNA ends (RACE; Frohman 1993) was performed. Using oligonucleotides M3 and KK1 (see materials and methods) derived from the central region of clone c9.1, the 3′ RACE product c9.17 and 5′ RACE product c9.28, respectively, were obtained. The clone c9.17 was located to the region 0.7–6.8 kb on the restriction map,

TABLE 6

Quantitation of the effect of *l(3)03834* in homozygous and heterozygous condition on gene expression in adults

Gene	*l(3)*/+ male	*l(3)*/*l(3)* male	*l(3)*/+ female	*l(3)*/*l(3)* female
white	1.15 ± 0.02*	1.27 ± 0.06*	0.64 ± 0.02*	0.65 ± 0.08*
Atu	0.98 ± 0.07	0.82 ± 0.12	0.89 ± 0.07	0.84 ± 0.13
Rga	0.45 ± 0.01*	—	0.50 ± 0.02*	—

Northern blots for each studied gene were performed in triplicate. The blots were quantitated after hybridization using a Fuji phosphorimager. The values obtained for each lane were divided by that of *rp49*, which was used as a control. The value for the *l(3)03834*/+ class was divided by the +/+ class and, in parallel, the value for *l(3)03834*^{rev}/+ was divided by the +/+ class. The ratio obtained for the *l(3)03834* class was normalized to that obtained for *l(3)03834*^{rev}. Finally, the value for the *l(3)03834*/*l(3)03834* class was divided by the *l(3)03834*/+ class and normalized to +/+ class. These ratios, obtained for each blot, were used for calculation of the mean and the standard errors. Those ratios marked with an asterisk are significantly different from 1 at the 95% confidence level in a *t* test.

thus comprising the cDNA c9.14, while cDNA c9.28 was mapped to the 7.0–7.6-kb area (Figure 1). The size of the composite cDNA was found to be 2.8 kb, which is in good agreement with the size of the detected mRNA (see above). The cDNA c9.14, corresponding to the 3′ end of the transcript, shows the same hybridization pattern as c9.1 when probed on a Northern blot (data not shown). Taken together, the data obtained from the cDNA and Northern analyses indicate that the cDNAs c9.14, c9.1, and the RACE products correspond to the same 2.8-kb mRNA.

Sequence analysis of the *Rga* transcript: The cDNAs, RACE products, and appropriate genomic fragment were sequenced on both strands. A derived composite 2.8-kb cDNA was found to have an open reading frame of 1740 bp. When compared to the genomic sequence, *Rga* cDNA reveals the presence of seven introns, with the longest (1445 bp) separating the untranslated exon I from exon II. The insertion of the *P* element in *l(3)03834* is located in exon I, and therefore before the translational start.

Conceptual translation of the open reading frame yields a predicted protein of 579 amino acid residues that has a molecular weight of ~59.4 kD (Figure 3A). The primary structure analysis reveals the presence of an *opa* or M repeat at position 61–71. This repeat is composed of $(CAA)_n$ or $(CAG)_n$, and it encodes a stretch of polyglutamine as it does in *Notch* (Wharton *et al.* 1985). Scattered polyglycine tracks were found within regions at positions 126–151 and 262–361.

A sequence database search using the BLAST program (Altschul *et al.* 1990) against GenBank sequences translated in the conceptual six frames failed to reveal any significant similarity. All matches resulted from the presence of polyglutamine and polyglycine tracks in

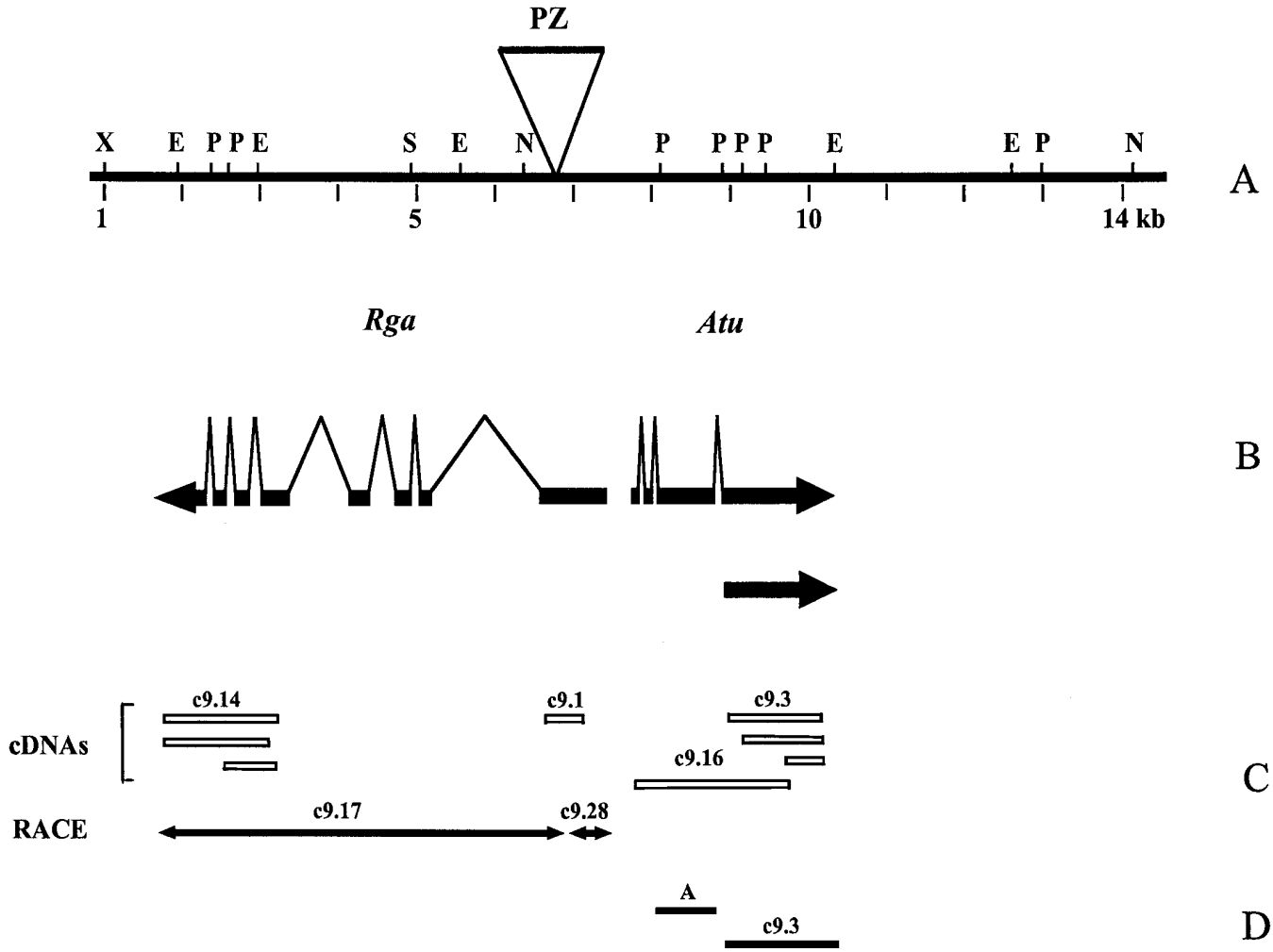

Figure 1.—Molecular map of the region containing the insertion site in *l(3)03834*. (A) Restriction map. Sites for endonucleases *Eco*RI (E), *Nhe*I (N), *Pst*I (P), *Sal*I (S), and *Xba*I (X) are shown. Site of the *P*[*lacZ, rosy*$^+$] insertion in stock *l(3)03834* is designated by a triangle. (B) Location, sizes, and intron–exon structure of the identified transcription units *Rga* and *Atu*. (C) Isolated cDNAs corresponding to *Rga* and *Atu* obtained by different methods: cDNAs isolated from cDNA libraries are shown by open boxes, and the RACE products for *Rga* mRNA are shown by lines with arrows on both sides. (D) Fragments of the *Atu* cDNA used for Northern analysis.

query. When the truncated RGA sequence without the 200 amino acid residues from the N-terminus containing these tracks was used in a similarity search, however, the homology to the yeast global negative transcription factor Cdc36p (Not2p; Collart and Struhl 1994) was found (Figure 3B). Even stronger homology was revealed to the *Caenorhabditis elegans* gene *BO286.4* (EMBL/GenBank accession number U39848). Two other homologues are human expressed sequence tags (ESTs; EMBL/GenBank accession numbers D17177 and H96804). Potentially due to the fact that ESTs were sequenced on one strand only, there are frameshifts in comparison to the predicted sequence of RGA.

Analysis of the *Atu* transcript: As mentioned above, in the course of cDNA library screens using the genomic fragments covering 14 kb around the insertion site, two classes of cDNAs were isolated, with one of them corresponding to the *Rga* gene (Figure 1). Another class of cDNA was found to map between positions 7.7 and 10.2 kb and defines *Atu* (*Another transcription unit*). Taking into account the close proximity of *Atu* and the *P*-element insertion site (Figure 1), the question was addressed whether the product of *Atu* could be responsible for the mutant phenotype of *l(3)03834*. To answer this question, a molecular characterization of *Atu* was also performed.

Deduced from DNA sequence analysis of overlapping cDNAs and genomic fragments, the *Atu* transcribed sequence consists of four exons and three small introns. Two nonoverlapping cDNA fragments were used for a Northern analysis. One of them is a cDNA c9.3, virtually corresponding to exon IV, while another one is fragment **A**, containing the sequences of exon III (Figure 1D). When the cDNA c9.3 (1.4 kb) was probed on a blot containing wild-type RNA, a minor (2.4 kb) and a major (1.4 kb) transcript were detected (Figure 2B). On the contrary, fragment **A** reveals only the 2.4-kb band and does not detect the 1.3-kb mRNA (Figure 2C). Thus, *Atu* produces two nested mRNAs overlapping in their respective 3′ ends.

The 5′ end of the 2.4-kb mRNA is located in close proximity to the *P*-element insertion site. To investigate whether it affects the transcription of the 2.4-kb mRNA, a Northern analysis in a segregating population of

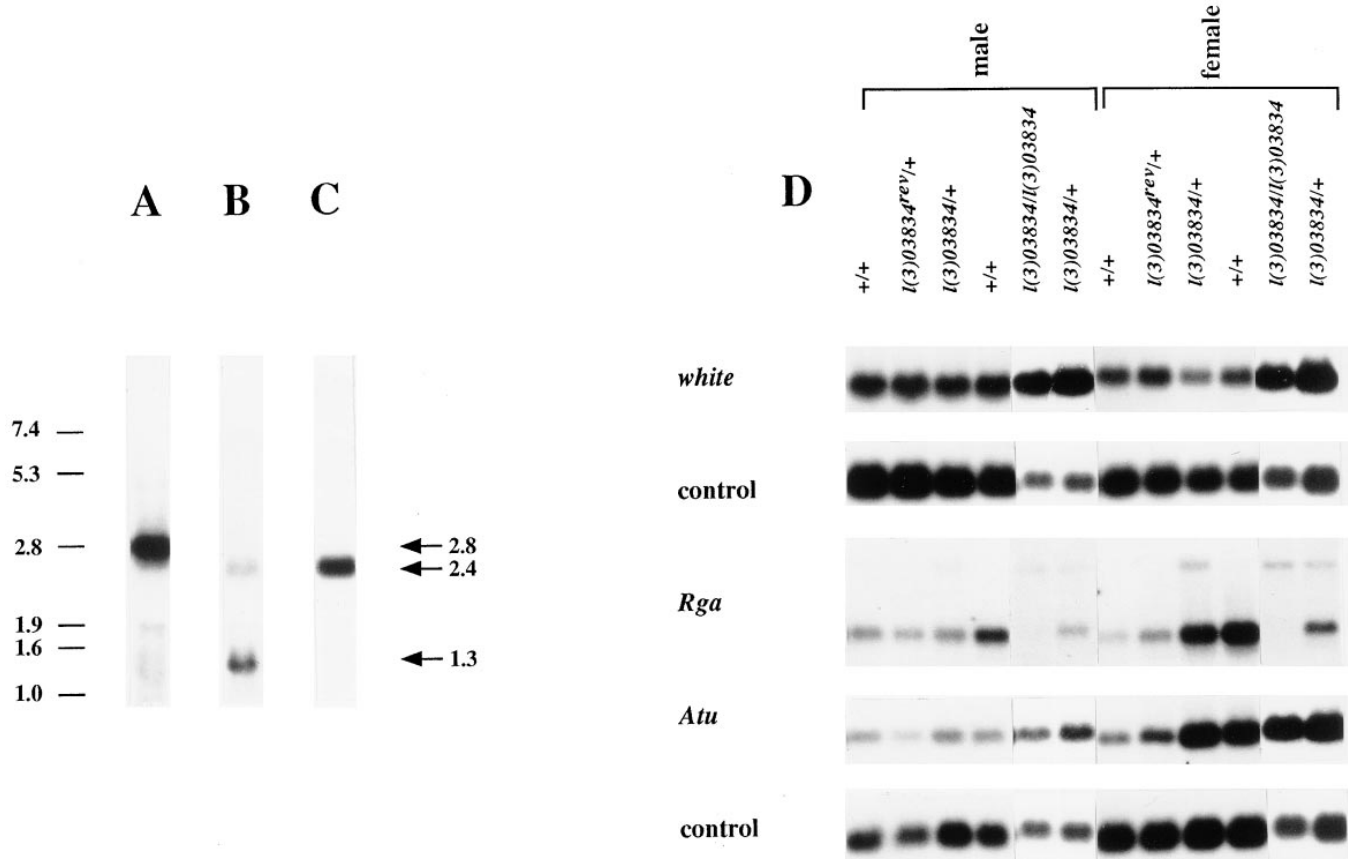

Figure 2.—Northern analysis of the region of *P*-element insertion in *l(3)03834*. (A–C) Hybridization of blot containing a wild-type RNA isolated from Canton S flies to antisense RNA probes synthesized from fragment of cDNA c9.1 (A), cDNA c9.3 (B), and fragment **A** of cDNA c9.16 (C). (D) Hybridization of a blot bearing total RNA from flies segregating for *l(3)03834* in males (lanes 3–6) and females (lanes 9–12) and *l(3)03834*^{rev} in males (lanes 1 and 2) and females (lanes 7 and 8) with antisense RNA probes for *white*, *Rga*, and *Atu*. Loading control (*rp 49*) is shown below.

A

```
MANLNFQQPPRSIANAALRGRTTGGFGGSSLAGHVTPTSGMFQTDFANSYPGTANYGQAPQQQQQQQQQP   70
QLSPNRNAQLSVGGPAISSGNRNANLFGQRQFVERRAMQGLGSGPMSNMGNFMQTGRGGYGTGGGGGGPL  140
NNFHVFGGGGGSDTSTPALLDPTEFPSLTNARGQNDQTLPQSNPLQPPGSKPYVGMVKQPTSEQSEFTMS  210
NEDFPALPGTQNSDGTTNAVGSVAGTGGSGGASTENHLDGTEKPMNSIVVSGSASGSSGSNVGVVGGNGL  280
GAVGSGIGGLAVGGGGGAGSSGGGGVGGNAASGVVGGSHVGLVGSNSGIGGVNSVPNSNAMMGVGGGLGS  350
GSGSSGSGAGGEHLNDNSSNDKLVKSGVQTSPDGKVTNIPATMVNNQFGMVGLLTFIRAAETDPNLVTLS  420
LGTDLTGLGLNLNSQESLHTTFAGPFVAQPCRAQDVEFNVPPEYLINFAIRDKLTAPVLKKLQEDLLFFL  490
FYTNIGDMMQLMAAAELHSREWRYHVEEKIWITRIPGIDQYEKNGTKERGTFYYFDAQSWKRLSKVFQID  560
PEKLDKCPNISAFMNGQSV                                                      579
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B

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RGA       GVQLSPDGKVTNIPATMVNNQFGMVGLLTFIRAAETDPNLV---TLSLGIDLTGLGLNLN-S----QESL 438
Human 1   -IQVLPDGRVTNIPQGMVTDQFGMIGLLTFIRAAETAQ                               37
Human 2   NI-------------------------------------------------------------------- 3
Nematode  GIILHPDGEVTNIPASMLDDQFGMASLVTYLRTVD-NPSIV---SLALGYDLTTLGLNLNLS----ERKL 224
Yeast     M------------------EKFGLKALVPLLKLEDKELSSTYDHSMTLGADLSSMLYSLGIPRDSQDHRV 52

RGA       HTTFAGPFVAQPCRAQDVEFNVPPEYL-INFAIRDKLTAP----------VLKKLQEDLLFFLFYTNIGD 497
Human 1                                                     37
Human 2   ---------------------------------HIRDKLAAI----------KLGRYGEDLLFYLYYMNGGD 31
Nematode  YMNFGGPWADSEIRAHELDVKVPEEYM-THNHIRDKLPPL----------RLNKVSEDVLFYLFYNCPNE 283
Yeast     LDTFQSPWAETSRSEVEPRFFTPESFTNIPGVLQSTVTPPCFNSIQNDQQRVALFQDETLFFLFYKHPGT 122

RGA       MMQLMAAAELHSREWRYHVEEKIWITRIPGIDQYEKNGTK-ERGTFYYFDAQSWKRLSKVFQIDPEKLDK 566
Human 1                                                     37
Human 2   VLQLLAAAVELFNRDWRYHKEERVWITRAPGMEPTMKTNTY-ERGTYYFFDCLNWRKVAKEFHLEYDKLEE 100
Nematode  IYQVAAACELYAREWRFHKSEQVWLTRSQYGGVKEQTGNY-PKGHYNVFDQMQWRKIPKELKLEYKELED 352
Yeast     VIQELTYLELRKRNWRYHKTLKAWLIKDPMMEPIVSADGLSERGSYVFEDPQRWEKCQRDFLL         185
```

Figure 3.—(A) Predicted protein sequence of RGA. (B) Multiple alignment of the predicted amino acid sequence of the C-terminal domain of RGA with that of the global-negative transcription factor Not2p from *Saccharomyces cerevisiae* (yeast); its homolog, gene B0286.4 (EMBL/GenBank accession number U39848), from *C. elegans* (nematode); and two translated human EST from *Homo sapiens* [EMBL/GenBank accession numbers D17177 (human 1) and H96804 (human 2)]. Amino acids that are identical between RGA and one or more of other proteins are shaded. The alignment was made by use of the computer program MegAlign from DNASTAR.

l(3)03834 was performed. As seen in Figure 2D and in Table 6, the transcription of the 2.4-kb *Atu* mRNA is not significantly affected in the case of the *P*-element insertion in both heterozygotes and homozygotes.

The derived composite *Atu* cDNA contains an open reading frame capable of encoding a protein of 724 amino acid residues (Figure 4). Several noteworthy features were found in the predicted amino acid se-

```
MGSQNSDDDSSSGSSRSGSRSVTPQGGSAPGSQRSRRSGSGSDRSRSGSRSSRSRSGSGSPRSARSGSAE   70
SRHSQLSASASRSKRSRSAHSRRSGSARTRKSGTPESPQSHRSGSLQSRKSGSPQSRRSGSPQSRKSGSTH  140
SRRSGSAHSRRSGSARSRKSGSAQSDRSESRSRSHSGSLKGNEESRSNSPNLQIDVERANSKSGSRSRSR  210
SRSGSRTSRSRSKTGTPSPNRSRSGSASGSGSDVGVPKKKARKASGSDQEKKKSGSDSDIEESPTKAKKS  280
RLIDTDSDSNQDVGKKAPAAADIFGDADDISDDEDEAGPAARKSPVRSKSRSQSKSHSHSRSMSHSRSRS  350
RSRSRDKVESQVESAPKEDEPEPLPETRIDVEIPRISADLGKEQHFIKLPNFLSVVTHPFDPETYEDEID  420
EEETMDEEGRQRIKLKVSNTIRWREYMNNKGDMVRESNARFVRWSDGSMSLHLGNEIFDAYRQPLLGDHN  490
HLFVRQGTGLQGQSVFRTKLTFRPHSTESFTHKKMTMSLADRSSKTSGIKILTQVGKDPTTDRPTQLREE  560
EAKLRQAMRNQHKSLPKKKKPGAGEPLIGGGTSSYQHDEGSDDENAISLSAIKNRYKKGSGAGQRAEVKA  630
STIYSSDEDEGSDFEARRSKKVDKAKASKALRDSDSESDAGSAKSGHSNKSGGEGGSASGSENEGSQKSG  700
GGSSKSASGSGSGSGSGSGSGSDND                                                725
```

Figure 4.—Predicted amino acid sequence derived from the composite cDNA of *Atu*. Nucleoplasmin-like nuclear localization signal (Robbins *et al.* 1991) is in italics. Multiple serine-arginine and arginine-serine repeats are underlined.

quence. First, a central portion of the protein between residues 395 and 595 reveals 27% identity to Leo1p from yeast (EMBL/GenBank accession number P38439; data not shown). The biological function of *LEO1* is unknown; however, its product was shown to be nonessential for cell viability, as indicated by the absence of a phenotype from gene disruption mutations (Magdolen *et al.* 1994). Second, the N-terminal 240 residues of ATU are 49% arginine plus serine. Many of these are present as arginine-serine or serine-arginine repeats. This feature is characteristic of an RS domain, which is found in a large group of related proteins that play an important role in spliceosome assembly and in 5′ splice site selection (for review see Horowitz and Krainer 1994; Fu 1995; Adams *et al.* 1996). The RS domain appears to participate in protein-protein interaction and is involved in directing the protein to nuclear speckles, the specific subnuclear compartment where splicing occurs (Li and Bingham 1991; Kohtz *et al.* 1994; for review, see Manley and Tacke 1996). It is unlikely, however, that the ATU protein is involved in splicing metabolism. Most proteins containing an RS domain also possess one or several RNA recognition motifs (Birney *et al.* 1993); the ATU protein does not. Also, ATU does not have the amino acid motifs that are necessary and sufficient for directing a protein to nuclear speckles. This motif comprises a nucleoplasmin-like nuclear localization signal and a repeating arginine-serine dipeptide sequence adjacent to a short stretch of basic amino acids (Hedley *et al.* 1995).

DISCUSSION

In this report, a newly recognized Drosophila gene, *Rga*, is described. It modulates *white* steady-state mRNA level differentially depending upon the developmental stage. In addition, it affects the transcript levels of several other related and unrelated genes, sometimes in a strongly sex-specific manner. *Rga* also acts as a suppressor of PEV. The data presented here demonstrate that these phenotypes result from a loss of function mutation of a gene containing a domain homologous to the yeast general transcription regulator *CDC36* (*NOT2*).

Initially, *Rga* was isolated in a large screen for autosomal recessive lethal *P*-element insertional mutations exhibiting an interaction with the *white* gene. A darker eye color was produced in the presence of an insertion on chromosome *3*, *l(3)03834*, and precise excision of the *P* element eliminated the eye color effects, lethality, and the other consequences as well.

There are two transcription units, *Rga* and *Atu*, located head to head near the *P*-element insertion site. Although the *P*-element insertion is near *Atu*, it is actually inserted in the transcription unit of the adjacent gene, *Rga*, and the normal transcription of *Atu* is not significantly affected by *l(3)03834*. This finding disfavors the possibility that *Atu* is responsible for the mutant phenotype in *l(3)03834*.

The data presented here argue that *l(3)03834* is a loss of function allele of the *Rga* gene. The insertion of the *P* element occurs within the 5′ untranslated region of this mRNA, thus disrupting its transcription. The level of normal *Rga* mRNA is decreased by one-half in *l(3)03834* heterozygotes and is not detected in mutant homozygotes, while the revertant has a normal level of these transcripts.

Several lines of evidence suggest that *Rga* encodes a protein involved in transcriptional regulation. The deduced amino acid sequence of RGA reveals a pronounced domain structure. The N-terminal domain is enriched in polyglutamine and is separated by polyglycine stretches from the NOT2-like, C-terminal domain. Polyglutamine repeats are considered to be common to transcription factors (Benedyk *et al.* 1994). They are found in both transcriptional repressors and activators, and their primary function is to interact specifically with other components of the transcriptional apparatus (for review, see Tjian and Maniatis 1994; Hanna-Rose and Hansen 1996). The C-terminal domain of the RGA protein shows significant similarity to the general negative regulator of transcription in yeast, Cdc36p (Not2p) (Collart and Struhl 1994). The Not2p-like domain is highly conserved, being present not only in yeast and Drosophila, but also in humans and nematodes. The polyglycine stretches located in the central portion of the protein potentially could serve as a hinge between the two domains, allowing flexibility in their relative positions.

The features attributed to the *Rga* gene seem to be rather unusual. Its loss of function allele exerts both positive and negative effects on the expression of the *white* gene, depending upon the developmental stage. Thus, increases or decreases of gene expression could be governed by a single gene. These developmental differences have been reported previously for three other *white* modifiers, *Inr-a* (Rabinow *et al.* 1991), *Wow* (Birchler *et al.* 1994), and *Mow* (Bhadra and Birchler 1996). However, *Rga* not only affects *white*, but also causes modulation of expression of several other tested genes. The most striking example is its strong sex-specific effect on the *X*-linked *Vinculin* gene. Although the transcription in males is normal, in females, it is severely decreased in the presence of the *Rga* mutation.

Perhaps the most remarkable feature of *Rga* is its direct or indirect involvement in regulation of chromatin function, which follows from its suppression of PEV. Three genes previously identified in a search for modifiers of *white* expression, *Wow* (Birchler *et al.* 1994), *Mow* (Bhadra and Birchler 1996), and *Low* (Bhadra *et al.* 1997a), were also recognized as weak modifiers of PEV. In addition, some genes isolated as modifiers of PEV are required for normal expression of homeotic genes. One of them, *Trithorax-like*, encodes the GAGA

transcriptional factor that binds to the promoters of several genes and stimulates their transcription (Farkas *et al.* 1994). Another example, *E(var)3-93D*, was also shown to display homeotic effects. It encodes a chromosomal protein with a domain homologous to several transcriptional regulators (Dorn *et al.* 1993). It was proposed that the latter two genes are involved in establishing or maintaining an open chromatin configuration that is required for transcriptional initiation.

There are two general models that could explain how *Rga* differentially regulates the transcription of many unrelated genes and suppresses PEV. *Rga* could interact with the components of the general transcriptional machinery. This mechanism was proposed for *CDC36* (*NOT2*) in yeast. *CDC36* (*NOT2*) has been suggested to act as a negative regulator that directly affects the utilization of the TATA element (Collart and Struhl 1994). But, unlike *CDC36* (*NOT2*), loss of function of *Rga* results in both negative and positive regulation of transcription of the target genes. This paradox could be resolved if *Rga* acts indirectly by affecting the expression of various other factors which, in turn, are involved in regulating gene activity both as repressors and activators. Among the loci regulated by *Rga* could be genes coding for components of heterochromatin, which could explain the suppression of PEV in the case of mutation in *Rga*.

In the second model, *Rga* acts on the establishment or maintenance of the appropriate chromatin state in the promoter region. Direct or indirect involvement of the RGA protein in regulation of chromatin structure follows from the finding that *Rga* acts as a weak suppressor of PEV. Since RGA does not possess any known DNA-binding motifs, however, it is unlikely to affect chromatin structure directly. Instead, it contains the glutamine-rich domain that could be responsible for protein-protein interactions (for review see Triezenberg 1995). Also, the Not2p-like domain, as proposed previously (Collart and Struhl 1994), could inhibit the basic transcription factor(s). One can suppose that *Rga* mediates the interaction between the components of chromatin and the RNA polymerase II transcriptional complex and/or transcriptional factors. On the other hand, it was demonstrated that the Not2p protein together with three other proteins, Not1p, Not3p, and Not4p, form a multiprotein complex in yeast (Collart and Struhl 1994). Homology searches against EST databases revealed the presence of both Not1p and Not4p homologs in humans, and Not4p in mice and nematodes. It is therefore likely that the Not proteins exist in Drosophila, and that RGA could interact with them in a complex via the glutamine-rich domain, for example. In this case, a putative Not4p containing two zinc fingers (Cade and Errede 1994; Irie *et al.* 1994) could mediate interaction between the RGA protein and the DNA of the promoter region of a target gene.

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