Mutation in \( P0 \), a Dual Function Ribosomal Protein/Apurinic/ Apyrimidinic Endonuclease, Modifies Gene Expression and Position Effect Variegation in Drosophila

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Manuscript received April 22, 1998
Accepted for publication August 24, 1998

ABSTRACT

In a search for modifiers of gene expression with the white eye color gene as a target, a third chromosomal \( P \)-element insertion mutant \( l(3)01544 \) has been identified that exhibits a strong pigment increase in a white-apricot background. Molecular analysis shows that the \( P \)-element insertion is found in the first intron of the gene surrounding the insertion site. Sequencing both the cDNA and genomic fragments revealed that the identified gene is identical to one encoding ribosomal protein \( P0/\)apurinic/apyrimidinic endonuclease. The \( P \)-element-induced mutation, \( l(3)01544 \), affects the steady-state level of \( white \) transcripts and transcripts of some other genes. In addition, \( l(3)01544 \) suppresses the variegated phenotypes of \( In(1)wm4h \) and \( In(1)y3P \), suggesting a potential involvement of the \( P0 \) protein in modifying position effect variegation. The revertant generated by the precise excision of the \( P \) element has lost all mutant phenotypes. Recent work revealed that Drosophila ribosomal protein \( P0 \) contains an apurinic/apyrimidinic endonuclease activity. Our results suggest that this multifunctional protein is also involved in regulation of gene expression in Drosophila.

EUKARYOTIC gene expression is thought to be organized into discrete steps such as transcription, splicing, and translation, with each step being performed by a separate group of proteins. However, over the past few years it has become obvious that there is a significant overlap among participants in these processes. This concept is supported by an increasing number of observations that some regulatory proteins are multifunctional and control more than one step of gene expression (reviewed in Ladomery 1997). One of the best-known examples is transcription factor IIIA (TF-IIIa). It was originally described as a transcription factor involved in \( 5S \) rRNA gene expression but was recently shown to be required for the packaging of \( 5S \) rRNA (Pieler and Theunissen 1993; Shastry 1996). Drosophila homeodomain protein, BICOID, previously has been seen as a transcription factor binding DNA and activating transcription of target genes. However, BICOID also appears to bind RNA in the 3'-untranslated region and to act as a translational repressor (Dubnau and Struhl 1996; Rivera-Pomar et al. 1996).

Human ubiquitous nuclear redox factor Ref-1 establishes the connection between transcription and DNA repair. Ref-1 regulates the DNA-binding activity of proto-oncogenes \( c-fos \) and \( c-jun \) by a post-translational mechanism involving reduction-oxidation. However, Ref-1 is a bifunctional protein as it also contains an apurinic/apyrimidinic (AP) endonuclease DNA repair activity and shows a significant homology to DNA repair enzymes from Drosophila and bacteria (Xanthoudakis et al. 1992).

Study of DNA repair pathways led to the discovery that transcriptionally active genes are preferentially repaired in mammalian cells (Mellon et al. 1987) and in bacteria (Mellon and Hanawalt 1989); therefore, DNA repair and transcription might be coupled cellular processes (for review see Drapkin et al. 1994; Lindahl et al. 1997). This concept is further supported by an increasing number of DNA repair proteins with a second function (reviewed in Lehmann 1998). One of them is the transcription factor TFIH, which assists other factors in loading RNA polymerase II onto DNA at the promoter site and promoter clearance (reviewed in Hoeijmakers et al. 1996). The largest subunit of TFIH was found to be mutated in a group of patients with defective nucleotide excision repair (Schaeffer et al. 1993).

In Drosophila, at least two ribosomal proteins, S3 and \( P0 \), are involved in DNA repair (Yacoub et al. 1996a, b). The S3 protein was shown to possess an activity toward 8-oxoguanine, a major form of DNA damage produced by oxidative stress (Yacoub et al. 1996a). The S3 also contains an AP lyase activity, cleaving phosphodiester bonds via a \( b,\delta \)-elimination reaction. Ribosomal phosphoprotein \( P0 \) acts on abasic DNA and shows strong DNase activity for both single- and double-stranded DNA (Yacoub et al. 1996b). Another dual-function ribosomal protein is S6, which is encoded by the gene abe-
rant immune response (air8). Mutations in the air8 gene cause melanotic tumors, and the flies display hypertrophied hemopoietic organs; therefore S6 was proposed to be required for tumor suppression in the hemopoietic system (Wat son et al. 1992).

Our lab is interested in building a hierarchy of the trans-acting modifiers that regulate the expression of a single-target locus in a dosage-dependent manner. The white locus provides a useful model system where these effects can be studied. Our interest in dosage-dependent modifiers is centered on the hypothesis that such modifiers 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).

In a search for modifiers of white gene expression we identified a gene encoding ribosomal protein P0. A P-element insertion in white mRNA at pupal stages, which correlates with the elevation of the steady-state level of white mRNA at these developmental stages. In contrast, a deletion within P0, apparently representing a null allele, decreases the level of white transcripts. In addition to white, the expression of Pgd and brown is affected by the mutation in P0. Finally, P0 acts as a suppressor of position effect variegation (PEV).

**MATERIALS AND METHODS**

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

A set of 508 single-autosomal P-element (Mlodzik and Hiro mi 1992) insertions from the Bloomington Stock Center was screened to identify the mutations affecting the eye color of white apricot flies. Among nine isolated mutations, l(3)01544, an insertion on chromosome 3 (79D1-2), was chosen for further analysis. To determine whether this insertion is responsible for the mutant phenotype, the P-element was mobilized by crossing l(3)01544/TM3, Sb flies to the delta 2-3, Sb/TM3, Ser strain (Robert son et al. 1988). The F1, l(3)01544/D 2-3, Sb males were crossed to the balancer stock TM3, Ser/M KRS. The Sb, non-Ser progeny (l(3)01544/M KRS) were screened for rosy flies, which were individually mated to TM3, Ser/M KRS to establish a stock.

For the developmental Northern analysis, genetic crosses were performed essentially as described (Birchler et al. 1994). Briefly, l(3)01544/TM3, Sb females were crossed to T(2;3)CyO, Cy Tb chromosomes. The F1 males containing this translocation heterozygous with l(3)01544 were mated to Canton-S females. The Tb marker allows discrimination between +/+ and l(3)01544/+ classes at the larval and pupal stages, while the Curly marker allows this distinction in adults.

**Pigment assay:** For eye-pigment assays, 1-day-old flies were separated according to their genotype and stored at ~80°C. To separate the heads from the bodies, flies were frozen in liquid nitrogen and vortexed in Eppendorf tubes for 1 min. Thirty heads per experiment were collected and the pigment assay was performed as described in Rab inow et al. (1991).

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

Genomic DNA was isolated from 50 flies by standard procedures with modifications described in Alatorcev (1988). The fragment flanking the insertion site in l(3)01544 was cloned by the “plasmid rescue” method according to Karpen and Spr ading (1992). The enzyme XbaI was used for digestion of 1 μg of the genomic DNA isolated from flies carrying the P-element insertion. After inactivation and dilution, a 200-μl ligation reaction was performed at 16°C overnight. The mixture was precipitated and used for electroporation of Escherichia coli DH5α.

The P1 phase 61-89, containing wild-type DNA from the 79D1-2 region on the cytological map (FlyBase http://flybase.bio.indiana.edu:82/), was used to obtain an overlapping set of fragments for cDNA library screens.

The cDNA library was prepared from 2-wk-old male and female wild-type adults (Canton-S) in the λZAP II vector (Stratagene, La Jolla, CA). About 600,000 phage have been screened as described in Sambrook et al. (1989).

Sequencing was performed on a Sequi-Gen GT nucleic sequencing cell (Bio-Rad, Richmond, CA) using the Sequenase (v2.0) kit (Amersham, Arlington Heights, IL). Homology searches were performed at the National Center for Biotechnology Information's BLAST WWW server.

To clone the region of the P-element insertion from the selected nine revertant stocks and from P0 and P0, the genomic DNA was isolated from homzygous flies and amplified by PCR using the primers 5'-CAGTTATGTACCCGAAAA TGCTCG-3' and 5'-CTTATTGCATCGAACGCTCAC-3'. The PCR fragments were cloned into pGEM-T vector (Promega, Madison, WI) and sequenced.

**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. Sequentially, 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. 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°C. For Northern analysis, three sets of RNA samples from the same isolation were prepared in running buffer (20 mm morpholino propan sulfonic acid, 5 mm sodium acetate, 1 mm EDTA) with 2.2 m formaldehyde and 50% (v/v) deionized formamide and then heat-denatured for 5 min at 65°C. 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 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, 8% dextran sulfate, 0.1 mg/ml heparin, 1% sarcosyl, 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°C.

Antisense RNA probes were synthesized with T3, T7, or SP6 RNA polymerase (Promega).

**RESULTS**

**Isolation and cloning of the P-element insertion l(3)01544:** We are interested in identifying a complete set of trans-acting modifiers regulating the expression of a single target gene, white. The leaky white apricot allele...
was used to screen a collection of lethal P-lacZ-element insertions (Karpen and Spradling 1992) for the presence of mutations exhibiting either elevated or decreased pigmentation intensity. The molecular basis of the \( w^1 \) lesion is an insertion of the retrotransposon, copia, into the second intron of white (Gehring and Paro 1980; Bingham and Judd 1981; O’Hare et al. 1984). The white-apricot allele was chosen because it produces a leaky, hypomorphic phenotype that allows detection of a wide range of modulation of white expression. Among several isolated insertional mutations, \((l(3)01544/TM3\) was selected for further study. The insertion maps to chromosome 3 at 79D1-2 and yields a darkened \( w^+ \) phenotype. To confirm that the observed phenotype is an effect upon white rather than one changing copia expression, the interaction of \((l(3)01544/TM3\) with different white alleles, such as point mutations, transposable element insertions, lesions in the structural and regulatory regions, and \( Adh \) promoter-white structural gene fusion construct, was examined. These tests were performed by crossing females carrying these white alleles to \((l(3)01544/TM3\) males. The eye color of the \((l(3)01544/TM3\) + males was compared to that of their \( TM3/TM3 \) brothers. From these crosses, one can gain information about whether the mutation affects the transcription of white or the process of pigment deposition itself or whether the effect is due to interaction with a transposable element in the white gene. The results of these tests are summarized in Table 1.

The insertions of the retroposon-I element, \( w^{111} \) and \( w^{55} \) (Sang et al. 1984), are not affected by \((l(3)01544/TM3\). On the contrary, the alleles, which are insertions of the retrotransposons copia \((w^6; Bingham and Judd 1981\) and blood \((w^6; Bingham and Judd 1981\), in the second intron of the white gene, are suppressed by \((l(3)01544/TM3\). Other suppressed alleles are the point mutations \( w^{52}, w^{35}, \) and \( w^d \) (Zachar and Bingham 1982), which implies that retroelements are not necessary for the interaction. The opposite effect was observed with four spotted alleles, \( w^b, w^{65}, w^{55}, \) and \( w^{81d5}\). These alleles represent lesions within the 5’ regulatory region that includes an eye enhancer (Zachar and Bingham 1982). Another two regulatory lesions are the reversions of the original \( w^1 \) mutation, \( w^h \) and \( w^e \). They are generated by secondary insertions into Doc (O’Hare et al. 1984), which causes the original \( w^1 \) mutation (Driver et al. 1989). Both \( w^h \) and \( w^e \) are not affected by \((l(3)01544/TM3\). Another nonresponding allele is \( w^i \), which is a duplica-

### Table 1

Effect of \((l(3)01544/TM3\) on selected alleles of the white gene

<table>
<thead>
<tr>
<th>Allele</th>
<th>Interaction</th>
<th>white locus lesion</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( w^1 ) (apricot)</td>
<td>+</td>
<td>copia retrotransposon insertion in intron 2</td>
<td>Gehring and Paro (1980); Bingham and Judd (1981)</td>
</tr>
<tr>
<td>( w^{111} )</td>
<td>None</td>
<td>I-element insertion</td>
<td>Sang et al. (1984)</td>
</tr>
<tr>
<td>( w^{55} ) (blood)</td>
<td>None</td>
<td>I-element insertion</td>
<td>Sang et al. (1984)</td>
</tr>
<tr>
<td>( w^i ) (ivory)</td>
<td>None</td>
<td>blood retrotransposon in intron 2</td>
<td>Zachar and Bingham (1982); Bingham and Chapman (1986)</td>
</tr>
<tr>
<td>( w^b ) (spotted)</td>
<td>−</td>
<td>B104 retrotransposon in 5’ regulatory region</td>
<td>Zachar and Bingham (1982); O’Hare et al. (1984)</td>
</tr>
<tr>
<td>( w^{65} ) (spotted-3)</td>
<td>−</td>
<td>Deficiency in 5’ regulatory region</td>
<td>Zachar and Bingham (1982)</td>
</tr>
<tr>
<td>( w^{55} ) (spotted-55)</td>
<td>−</td>
<td>mdg3 retrotransposon in 5’-untranslated leader</td>
<td>Zachar and Bingham (1982)</td>
</tr>
<tr>
<td>( w^{61d5} ) (spotted-81d5)</td>
<td>−</td>
<td>Deficiency in 5’ regulatory region</td>
<td>Davison et al. (1985)</td>
</tr>
<tr>
<td>( w^{12} ) (apricot-2)</td>
<td>+</td>
<td>Point</td>
<td>Zachar and Bingham (1982)</td>
</tr>
<tr>
<td>( w^{77} ) (carrot)</td>
<td>+</td>
<td>Point</td>
<td>Zachar and Bingham (1982)</td>
</tr>
<tr>
<td>( w^{35} ) (coffee)</td>
<td>+</td>
<td>Point</td>
<td>Zachar and Bingham (1982)</td>
</tr>
<tr>
<td>( w^{55} ) (colored)</td>
<td>+</td>
<td>Point</td>
<td>Zachar and Bingham (1982)</td>
</tr>
<tr>
<td>( w^i ) (honey)</td>
<td>None</td>
<td>B104 element into Doc element of ( w^i )</td>
<td>O’Hare et al. (1991)</td>
</tr>
<tr>
<td>( w^e ) (eosin)</td>
<td>None</td>
<td>Transposable element reversion of ( w^i ) (Doc element)</td>
<td>Zachar and Bingham (1982); O’Hare et al. (1984); Hazelrigg (1987)</td>
</tr>
<tr>
<td>z Dp(1;1)w +51d5</td>
<td>None</td>
<td>Duplicated white locus sequences</td>
<td>Gunaratne et al. (1986)</td>
</tr>
<tr>
<td>Adh-w #2</td>
<td>None</td>
<td>Adh promoter-white structural gene on chromosome 3</td>
<td>Birchler et al. (1990)</td>
</tr>
</tbody>
</table>

Females carrying the various X-linked white alleles were crossed to males carrying \((l(3)01544/TM3\) balancer chromosome marked by Sb. \((l(3)01544/TM3\) + males were then compared to their \( TM3/TM3 \) + brothers. −, enhancement; +, suppression.
tion of sequences of the first intron to the start of exon 3 (Kar ess and Rubin 1982). l(3)01544 shows no effect when the z1 mutation represses white expression. Finally, no response was found for the Adh promoter-white structural gene construct (Bir ch ler et al. 1990). Thus, the allele specificity tests reveal the necessity of the white regulatory sequences in order for interaction with l(3)01544 to occur.

The genomic sequence flanking the P-element insertion was cloned via the “plasmid rescue” method and used as a probe to isolate an overlapping set of genomic fragments covering ~20 kb from the insertion site. Screening a random and oligo(dT)-primed cDNA library yielded five independent clones that were mapped to the region 0.9–2.3 kb on the restriction map, thus covering the position of the P-element insertion (Figure 1). No cDNAs mapped outside this region were found. The largest cDNA, c7.1, was sequenced on both strands while four others were partially sequenced and their respective 3′ and 5′ sequences were found to overlap with portions of c7.1. When c7.1 was used as a probe on Northern blots of wild-type RNA, an abundant 1.3-kb transcript was detected, the length being in accordance with the mRNA revealed by cDNA c7.1. It was therefore concluded that l(3)01544 is a mutation in the P0 gene.

To demonstrate that the eye-color phenotype and lethality attributed to l(3)01544 are caused by the same insertion, the P element was mobilized by supplying transposase with the A2-3 chromosome (Roberston et al. 1988). Genomic DNA isolated from 200 independent rosy− stocks was subjected to Southern analysis. In brief, 99 stocks retain a portion of the P element, implying that internal deletions within the P element took place, while one, namely P036, was found to be a 0.5-kb deletion removing the sequences of the 5′ region of the P0 gene (Figure 1). Three quarters of these stocks, 77%, were lethal as homozygotes and exhibit an interaction with w+, while the remaining 23% show a complete reversion of the mutant phenotype. These 23% of derivatives, in particular P08 and P036, were found to contain a short fragment of the P transposon. The remaining 100 stocks appear to be precise excisions and 9 of them were analyzed in more detail. All 9 chromosomes were viable as homozygotes and condition no eye-color phenotype. Using PCR with specific oligonucleotide primers derived from the genomic sequence of P0 on both sides of the P-element insertion site, the DNA fragments from each of the 9 selected revertants and from P08 and P036 were amplified, cloned, and sequenced. It was therefore confirmed that P08 and P036 are internal deletions of the P element that have remaining variable lengths of terminal-inverted repeat from the 5′ and 3′ ends flanked by the 8-bp duplication. In the stocks P08 and P036, 45 and 55 bp, respectively, of residual P-element sequences remain in the first intron of the P0 gene. On the contrary, in each of the other revertants the excision is accompanied by loss of the P-element inverted terminal repeats and one copy of the 8-bp duplication, thus restoring the wild-type sequence. One of the stocks with the precise excision of the P element, P010, was chosen for further study as a control for normal expression of the P0 gene (see below). Taken together, these results indicate that the insertion of this P element into the P0 gene causes both the lethality and the eye-color phenotype.

Effect of the mutant allele P0(l(3)01544) on the transcript abundance of unrelated genes: Because P0(l(3)01544) modifies the eye color of whiteapricot flies, its effect upon white transcripts was examined. To study the effect in pupae, the stage during which the majority of pigment is deposited, crosses for developmental Northern analysis were performed as described in materials and methods. Triplicate Northern blots containing total RNA of developmentally staged samples collected from mid-
and late-stage pupae were hybridized with white and P0 antisense probes. The same blots were then probed with rRNA used as a gel-loading control. The results of phosphorimager scans are given in Table 2. Surprisingly, the mutation results in apparent elevation of P0 transcripts in midpupal males and in late pupal males and females. The amount of white transcripts was also found to be significantly increased in males. This elevation correlates with the phenotypic effect. Hence, the level of white expression responds directly to an increase in the level of P0.

To understand whether expression of other genes besides white is affected, the steady-state level of two related genes, brown (bw; Dr esse n et al. 1988) and scarlet (st; Tearle et al. 1989), as well as four unrelated genes, rudimentary (r; S e graves et al. 1984), Phosphogluconate dehydrogenase (Pgd; Scott and Lucchesi 1991), β-tubulin (β Tub6D; Bial o jan et al. 1984), and ribosomal protein 49 (rp49; Kong suwan et al. 1985) were examined. RNA isolated from larvae segregating for P0301544 (see materials and methods) was subjected to Northern analysis. Triplicate RNA transfers were hybridized with the probes of the genes mentioned above and with the rRNA probe as a gel-loading control. The results of phosphorimager scans are shown in Table 3. Briefly, among seven tested genes, four genes (st, r, rp49 and β-tubulin) did not respond to P0301544. The response of three other genes is either sex-specific or independent of sex. The steady-state level of w and bw is elevated and no differences were found between males and females. In contrast, the transcripts of Pgd are increased in females only. Thus, the observation that, in addition to white, the mutant allele of P0301544 exerts an effect on the steady-state RNA level of two of six tested genes implies that the product of the P0 gene may be involved in general regulation of gene expression.

### TABLE 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midpupae</td>
<td>1.76 ± 0.12*</td>
<td>2.19 ± 0.23*</td>
</tr>
<tr>
<td>Male</td>
<td>0.83 ± 0.08</td>
<td>0.65 ± 0.07*</td>
</tr>
<tr>
<td>Late pupae</td>
<td>3.36 ± 0.12*</td>
<td>2.33 ± 0.12*</td>
</tr>
<tr>
<td>Female</td>
<td>1.09 ± 0.02*</td>
<td>1.20 ± 0.00*</td>
</tr>
</tbody>
</table>

Northern blots for each developmental stage were performed in triplicate. The blots were quantitated using a Fuji phosphorimag er. The values obtained for each lane were divided by the value of rRNA used as a control. The value for the P0301544 + class was divided by the +/+ class. These ratios were used for calculation of the mean and standard errors for the three replicates. * The P0301544 + class that is significantly different from the +/+ class at the 95% confidence level in a t-test.

### TABLE 3

<table>
<thead>
<tr>
<th>Gene</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>white</td>
<td>1.23 ± 0.03*</td>
<td>1.51 ± 0.09*</td>
</tr>
<tr>
<td>brown</td>
<td>1.60 ± 0.11*</td>
<td>2.08 ± 0.15*</td>
</tr>
<tr>
<td>scarlet</td>
<td>1.05 ± 0.09</td>
<td>1.04 ± 0.06</td>
</tr>
<tr>
<td>rudimentary</td>
<td>1.06 ± 0.03</td>
<td>1.06 ± 0.10</td>
</tr>
<tr>
<td>Pgd**</td>
<td>0.85 ± 0.09</td>
<td>2.38 ± 0.11*</td>
</tr>
<tr>
<td>β-Tubulin</td>
<td>1.10 ± 0.03</td>
<td>1.04 ± 0.05</td>
</tr>
<tr>
<td>rp49</td>
<td>0.98 ± 0.06</td>
<td>1.07 ± 0.03</td>
</tr>
</tbody>
</table>

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 the value of rRNA used as a control. The value for the P0301544 + class was divided by the +/+ class. These ratios, obtained for each blot, were used for calculation of the mean and standard errors. * Ratios significantly different from 1 at the 95% confidence level in a t-test. ** Ratios have a level of expression in females significantly different from that in males at the 95% confidence level in a t-test.

Data from Northern analyses described above imply a direct correlation between the level of P0 and white transcripts; hence, the P-element-induced mutation P0301544, which results in an elevation of P0 levels, is a hypermorphic allele. If this is the case, then a deletion, P0306, should decrease the level of P0 and white expression. To test this assumption, Northern blots of total RNA isolated from adults of a segregating population of P0301544 and that of P0306 were made. As a control, the revertant allele P0rev was used. In the case of the P-element insertion, the steady-state levels of both P0 and white transcripts are increased in females, while males show a reduction of the P0 expression (Table 4). On the contrary, flies carrying the deletion P0306 showed the P0 mRNA to be decreased to one-half relative to the respective controls in both males and females. In turn, the steady-state level of white mRNA is slightly but significantly decreased in both sexes. The transcript level in the revertant P0rev is restored to normal. Thus, P0301544 is a gain-of-function allele and white shows a positively correlated response to the dosage of P0.

**Effect of P0 on PEV:** Four genes previously identified as modifiers of white expression were also found to be suppressors of PEV (Birchler et al. 1994; Bhadra and Birchler 1996; Bhadra et al. 1997a; Frolov et al. 1998). We addressed the question of whether mutant alleles of P0 affect PEV in a rearrangement chromosome, In(1)wm4h. 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 position effect variegation (Henikoff 1996). This test was performed by crossing In(1)wm4h females to males carrying mutant alleles of P0. P0301544 was found to suppress...
of a preexisting mutation on the P0\textsuperscript{[1]l(3)01544} chromosome that suppresses PEV, different from the P-element insertion, the same analysis was done for revertant flies. It was found that the revertant P0\textsuperscript{rev} no longer suppresses PEV (Table 5). In contrast, the deletion P0\textsuperscript{[1]l(3)01544} shows no change in pigment level when In(1)\textsuperscript{wmb} is present (Table 5).

To test whether the suppressing effect of P0\textsuperscript{[1]l(3)01544} on the In(1)\textsuperscript{wmb} chromosome is due to a general suppression of PEV rather than a specific interaction with the white gene, the effect of P0\textsuperscript{[1]l(3)01544} on a variegating allele of yellow in the inversion In(1)y\textsuperscript{3P} was examined. For segregating classes, P0\textsuperscript{[1]l(3)01544}/TM3 males were crossed to In(1)y\textsuperscript{3P} females, and the F1 males segregating for P0\textsuperscript{[1]l(3)01544} and the TM3 balancer were scored. The number of wild-type and yellow triple-row bristles along the anterior margin of the wing blades was counted in each class of flies (Table 6). P0\textsuperscript{[1]l(3)01544} reduces the frequency of yellow variegation among the In(1)y\textsuperscript{3P} flies, thereby suppressing the yellow bristle variegation nearly twofold (10.9%) above the comparable control values (21.8%). On the contrary, the revertant flies show no suppression of yellow variegation. Thus, P0\textsuperscript{[1]l(3)01544}, but not the revertant, suppresses variegation of both white and yellow in two different rearrangements, implying that P0 is a general modifier of PEV.

**DISCUSSION**

In a search for modifiers of white gene expression, the lethal P-element insertional mutation, l(3)01544, which darkens the eye color of whiteapricot flies, was recovered. Several lines of evidence suggest that l(3)01544 is the P-element mutation in the gene-encoding ribosomal protein P0 and the insertion causes the mutant phenotype. First, molecular analysis of l(3)01544 revealed that the insertion occurs within the first intron of the transcription unit located nearby. The amino acid sequence derived from the cDNA exactly matches that of ribosomal protein P0 whose cytological localization coincides with the site of the P element (Kelley et al. 1989). Second, both the identified gene and P0 produce an mRNA of the same size. Third, P0 is a single-copy gene based on Southern blot analysis (Kelley et al. 1989; our

**TABLE 4**

Quantitation of white and P0 transcripts from segregating P0\textsuperscript{306}, P0\textsuperscript{l(3)01544}, and P0\textsuperscript{rev} populations in adults

<table>
<thead>
<tr>
<th>Allele</th>
<th>white</th>
<th>P0</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0\textsuperscript{306}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.64 ± 0.06*</td>
<td>0.51 ± 0.02*</td>
</tr>
<tr>
<td>Female</td>
<td>0.80 ± 0.07*</td>
<td>0.47 ± 0.03*</td>
</tr>
<tr>
<td>P0\textsuperscript{l(3)01544}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.07 ± 0.04</td>
<td>0.80 ± 0.02*</td>
</tr>
<tr>
<td>Female</td>
<td>2.03 ± 0.09*</td>
<td>1.47 ± 0.02*</td>
</tr>
<tr>
<td>P0\textsuperscript{rev}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.10 ± 0.07</td>
<td>1.12 ± 0.06</td>
</tr>
<tr>
<td>Female</td>
<td>0.90 ± 0.05</td>
<td>1.05 ± 0.03</td>
</tr>
</tbody>
</table>

Northern blots for each P0 allele were performed in triplicate. The blots were quantitated using a Fuji phosphorimager. The values obtained for each lane were divided by the value of rRNA used as a control. The value for the P0\textsuperscript{+} class was divided by the +/- class. These ratios were used for calculation of the mean and standard errors for the three replicates. * The P0\textsuperscript{+} class is significantly different from the +/- class at the 95% confidence level in a t-test.

In males of each genotype in triplicate. As a control the same values were calculated for the P0\textsuperscript{rev} flies. * The value is significantly different from the control at the 95% confidence level in a t-test.

**TABLE 5**

Pigment assay results of the effect of P0\textsuperscript{l(3)01544}, P0\textsuperscript{306}, and P0\textsuperscript{rev} on In(1)\textsuperscript{wmb} in males

<table>
<thead>
<tr>
<th>Genotype</th>
<th>OD\textsubscript{400}</th>
</tr>
</thead>
<tbody>
<tr>
<td>w\textsuperscript{mb}; P0\textsuperscript{l(3)01544}/+</td>
<td>0.317 ± 0.016*</td>
</tr>
<tr>
<td>w\textsuperscript{mb}; TM3/+</td>
<td>0.093 ± 0.018</td>
</tr>
<tr>
<td>w\textsuperscript{mb}; P0\textsuperscript{101}/+</td>
<td>0.052 ± 0.011</td>
</tr>
<tr>
<td>w\textsuperscript{mb}; M KRS/+</td>
<td>0.044 ± 0.008</td>
</tr>
<tr>
<td>w\textsuperscript{mb}; P0\textsuperscript{rev}/+</td>
<td>0.047 ± 0.008</td>
</tr>
<tr>
<td>w\textsuperscript{mb}; M KRS/+</td>
<td>0.046 ± 0.007</td>
</tr>
</tbody>
</table>

The effect of P0\textsuperscript{l(3)01544} and P0\textsuperscript{rev} on In(1)\textsuperscript{wmb} is determined from the OD\textsubscript{400} values, which were measured in the eyespigment assay on males of each genotype in triplicate. As a control the same values were calculated for the P0\textsuperscript{rev} flies. * The value is significantly different from the control at the 95% confidence level in a t-test.

**TABLE 6**

The effect of P0\textsuperscript{l(3)01544} on yellow variegation in In(1)y\textsuperscript{3P}

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total number of bristles</th>
<th>Frequency of yellow triple-row bristles (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>y\textsuperscript{3P}; P0\textsuperscript{l(3)01544}/+</td>
<td>1873</td>
<td>10.9*</td>
</tr>
<tr>
<td>y\textsuperscript{3P}; TM3/ +</td>
<td>1982</td>
<td>21.8</td>
</tr>
<tr>
<td>y\textsuperscript{3P}; P0\textsuperscript{rev}/+</td>
<td>1630</td>
<td>18.7</td>
</tr>
<tr>
<td>y\textsuperscript{3P}; M KRS/+</td>
<td>1725</td>
<td>19.8</td>
</tr>
</tbody>
</table>

* The value is significantly different from the control at the 95% confidence level.
unpublished observations). Fourth, the darker eye color phenotype of \( P^{0(3)01544} \) is apparently associated with the insertion because precise excision completely eliminates the lethality and the mutant phenotype.

As further evidence of participation of P0 in regulation of gene expression, a direct correlation between the level of P0 and white expression was found. The deletion P0\(^{06}\), apparently representing a null allele, causes the level of P0 transcripts to be decreased to one-half in heterozygotes and the expression of the white gene is decreased as well. On the contrary, the allele P0\(^{(13)01544}\) elevates the steady-state level of P0 expression in late pupae and, in turn, the expression of the white gene is increased. In the revertant generated by the precise excision of the P element, both P0 and white transcripts return to the normal level. Therefore, one can assume a dosage effect of P0 upon the expression of the target gene, white. It is still not clear how an insertion into the transcription unit in the allele P0\(^{(13)01544}\) could result in an elevation of the expression of the disrupted gene. One possibility is that the insertion could change the developmental pattern of expression and induce the expression of the P0 gene in tissues or primodia where normally it is not transcribed. A similar effect was found in the case of Mutator insertions into the fifth intron of the maize knotted1 gene. The insertions result in ectopic expression within developing leaves where the gene is not normally expressed (Smith et al. 1992; Greene et al. 1996).

The P0 gene was initially cloned by screening a cDNA expression library with an antibody against major human AP endonuclease (Kelley et al. 1989). Surprisingly, the identified Drosophila protein was 79% similar in amino acid sequence to that of human ribosomal protein P0 (Grabowski et al. 1991). However, further biochemical analysis demonstrated an associated AP endonuclease activity that is specific to AP sites in DNA (Yacoub et al. 1996b). Moreover, when the P0 gene was transformed in an E. coli strain deficient for the 5′-acting endonucleases, it was able to reverse the sensitivity of mutant cells to an alkylating agent known to produce homodimers of two other ribosomal proteins, P1 and P2. Thus, the P0 gene apparently represents a multifunctional protein involved in both DNA repair and protein translation. Another example linking these two processes is Drosophila ribosomal protein S3, which contains 8-oxoguanine and abasic site DNA repair activities (Yacoub et al. 1996a).

How could the P0 protein with DNA repair activity participate in the regulation of gene expression in Drosophila? P0 is associated with ribosomes and was also found in the nuclear matrix where it is involved in DNA repair metabolism (Yacoub et al. 1996b). This does not exclude the possibility that, besides DNA repair, P0 directly participates in other processes related to gene expression. Perhaps each function attributed to P0 is catalyzed by a different domain as found for a major human AP endonuclease, Ape. Ape is a multifunctional protein that was shown to be the transcription factor Ref-1 regulating Fos and jun binding to DNA via a redox mechanism (Xanthoudakis et al. 1992). In addition, Ref-1 stimulates the binding of p53 to DNA in both a redox-dependent and a redox-independent manner, implying that it has a third distinctive function in the cell (Jayaraman et al. 1997). Sequence analysis revealed that Ape has a central core that is highly homologous to other prokaryotic and eukaryotic AP endonucleases, while the N-terminal domain does not reveal any similarities to known protein sequences. The N-terminal sequences of Ref-1 were dispensable for the AP endonuclease activity but required for redox activity (Xanthoudakis et al. 1992).

Another Drosophila DNA repair gene, mus209, encodes a proliferating cell nuclear antigen (PCNA; Henderson et al. 1994) that is an auxiliary factor of DNA polymerases δ and ε, ensuring processivity of replication (Kelzman 1997). Homozygous flies mutant for mus209 were found to suﬀer PEV (Henderson et al. 1994). Interestingly, in addition to P0, four genes previously isolated in screens for modifiers of white are weak modifiers of PEV (Birchler et al. 1994; Bhadra and Birchler 1996; Bhdara et al. 1997a; Frolov et al. 1998). Perhaps the suppression of PEV by the hypermorphic allele P0\(^{(13)01544}\) could give some clues to the mechanism of P0 action. If P0 is tightly associated with the nuclear matrix (Yacoub et al. 1996b) and prevents the local formation and/or spreading of heterochromatin, then an increased level of P0 found in P0\(^{(13)01544}\) would suppress PEV.

Alternatively, P0 could act indirectly as a ribosomal protein through alteration of translation of various factors that are involved in regulation of gene expression. The observation that the expression of several tested genes was not affected by the mutation in P0 could imply that P0 regulates translation of a specific subset of cellular mRNAs. Study of mammalian ribosomal proteins revealed that a single copy of P0 is integrated with homodimers of two other ribosomal proteins, P1 and P2, into a coherent structure, P1-P2-P0, which is a component of the large ribosomal unit (reviewed in Wool et al. 1995). The core particles depleted of P-proteins are unable to catalyze protein synthesis; they do not associate with EF-1 and EF-2. Hence, one would expect that mutation in the P0 gene would result in a general reduction in translational efficiency if it were rate limiting rather than a reduction of translation of particular classes of mRNAs. Thus, this alternative seems less likely.

The so-called “Minute” phenotype is characterized by reduced body size, rough eyes, reduced viability, deformed or otherwise affected antennae, and recessive lethality (Lindsley and Zimm 1992). The Minute phenotype is thought to be associated with mutations in ribosomal genes (Lindsley and Zimm 1992), although
some exceptions have been reported. For example, genes encoding ribosomal proteins L1 (Raftí et al. 1988) and RP17 (McNabb and Ashburner 1993) map to the regions where a Minute has not been identified. It should be noted that we did not observe a Minute phenotype in either P0 or P0 (l(3)01544) mutant flies. Also no Minute mutation has been mapped to the region of P0 (Lindsley and Zimm 1992). One possible explanation is that the expression of P0 is not haplo-insufficient for its ribosomal function. On the other hand, P0 is haplo-insufficient for its effect on gene expression. Therefore, an involvement of P0 in regulation of gene expression could be distinct from its role as a ribosomal protein.

P-proteins are unusual among ribosomal proteins in that they show phosphorylation of serine residues at the C terminus by different protein kinases in response to various stimuli (reviewed in Wool et al. 1995). Phosphorylation of P-proteins is necessary for their assembly into large ribosomal subunits; dephosphorylated P-proteins lose their binding ability and they dissociate from the ribosome (Sanchez-Madrid et al. 1981; reviewed in Wool et al. 1995). Another ribosomal protein exhibiting a developmentally regulated phosphorylation is S6. Mutation in Drosophila S6 leads to abnormal blood cell differentiation and melanotic tumor formation in larvae (Watson et al. 1992). Phosphorylation of S6 in mammalian cells is thought to play a central role in coordinate translational upregulation of transcripts of ribosomal genes (Jefferies et al. 1997). Both S6 and P0 are overexpressed in human colon carcinomas and liver metastases (Barnard et al. 1992). Our finding that P0 is involved in modulation of gene expression reveals another extraribosomal regulatory function of this ribosomal protein.

We thank the Indiana University Drosophila Stock Center for providing flies of l(3)01544, Dan Hartl for sending the P1 clone, and Mark Kelley for helpful discussion and communication of results. Special thanks to members of the Birchler lab for discussion and critical comments. This study was supported by National Science Foundation grant to J.A.B. M.V.F. was supported by a postdoctoral fellowship to the Molecular Biology Program at the University of Missouri, Columbia.

LITERATURE CITED


