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$TAF_{II}40$ Protein Is Encoded by the *e(y)1* Gene: Biological Consequences of Mutations

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The *enhancer of yellow 1* gene, *e(y)1*, of *Drosophila melanogaster* has been cloned and demonstrated to encode the $TAF_{II}40$ protein. The *e(y)1* gene is expressed in females much more strongly than in males due to the accumulation of *e(y)1* mRNA in the ovaries. Two different *e(y)1* mutations have been obtained. The $e(y)1^{ul}$ mutation, induced by the insertion of *Stalker* into the coding region, leads to the replacement of 25 carboxy-terminal amino acids by 17 amino acids encoded by the *Stalker* sequences and to a decrease of the *e(y)1* transcription level. The latter is the main cause of dramatic underdevelopment of the ovaries and sterility of females bearing the *e(y)1* mutation. This follows from the restoration of female fertility upon transformation of $e(y)1^{u1}$ flies with a construction synthesizing the mutant protein. The $e(y)1^{P1}$ mutation induced by *P* element insertion into the transcribed nontranslated region of the gene has almost no influence on the phenotype of flies. However, in combination with the ph^{P1} mutation, which leads to a strong *P* element-mediated suppression of *e(y)1* transcription, this mutation is lethal. Genetic studies of the $e(y)1^{u1}$ mutation revealed a sensitivity of the *yellow* and *white* expression to the $TAF_{II}40$/e(y)1 level. The su(Hw)-binding region, *Drosophila* insulator, stabilizes the expression of the *white* gene and makes it independent of the $e(y)1^{u1}$ mutation.

Initiation of transcription by RNA polymerase II requires an ordered assembly of a multiprotein preinitiation complex at the core promoter of eucaryotic genes (56, 58). TAFs (TATA-binding protein-associated factors), which are highly conserved in all organisms from yeasts to mammals, are the components of the TFIID complex of the basal transcription machinery (11). TAFs are considered to perform important functions both in transcription and in core promoter recognition (46, 47). Some TAFs can function as coactivators and mediate activation signals from enhancer-bound regulatory proteins (7, 8, 14, 15, 28, 33, 59).

While TFIID has been extensively studied in vitro, very little is known about the function of individual TAFs in vivo. Studies with yeasts demonstrated that the absence of several TAFs did not influence the overall level of transcription but led to the death of cells, associated with specific cell cycle arrest phenotypes (2, 40, 61, 63). It has been determined that transcription of some yeast genes depends on $TAF_{II}145$ (62). Results of studies of higher-eucaryotic TAFs are consistent with these results. Mutations in genes for two highly conserved TAFs, $TAF_{II}60$ and $TAF_{II}110$, reduced transcription of *Bicoid*-dependent target genes in *Drosophila* embryos (52) and led to lethality at the embryonic stage.

$TAF_{II}40$ of *Drosophila melanogaster* ($dTAF_{II}40$) is a member of the TAF family that has homologues in other higher eucaryotes (28). Several studies of the $TAF_{II}40$ function in vitro were performed. A protein-protein interaction assay revealed direct binding between $TAF_{II}40$ and the activation domains of VP16 (28) and p53 (57). A human homologue of $dTAF_{II}40$, $hTAF_{II}31$, was also identified as a critical protein required for p53 (38)- and VP16 (35)-dependent activation of transcription. The $TAF_{II}40$ protein was postulated to mediate the activation by proteins with acidic domains. $TAF_{II}40$ and $TAF_{II}60$ were shown to contain histone folding motifs and to cocrystallize in a histone-like structure (30, 64). Although in vitro results suggest that $TAF_{II}40$ plays an important role in transcription, no studies of $TAF_{II}40$ function have been performed in vivo.

In our previous works, we identified mutations in the *e(y)1*, *e(y)2*, and *e(y)3* genes (19, 20) that enhanced the phenotype of the y^2 mutation. It was suggested that the protein products of these genes performed general and related functions in the regulation of transcription. They are involved in the activation of several genes and cooperate with the zeste protein in the control of *white* gene expression (21). Combinations of weak mutations of these genes are lethal.

In this study, we have cloned the *e(y)1* gene and found that it encodes $dTAF_{II}40$. Two *e(y)1* mutations have been described. $TAF_{II}40$ has been demonstrated to be indispensable. It accumulates in ovaries, and the inhibition of *e(y)1* transcription severely suppresses oogenesis. The expression of at least a certain group of genes has been shown to be sensitive to a partial inhibition of *e(y)1* transcription.

MATERIALS AND METHODS

Genetic crosses. Flies were cultured at 25°C in standard *Drosophila* wheat meal-yeast-sugar-agar medium. All crosses were performed in standard glass vials with 5 to 10 males and 10 to 15 females per vial. The origin of $e(y)1^{u1}$ and $e(y)1^{P1}$ [$e(y)4^{P1}$] alleles, mutations and constructions used in this work were described elsewhere (17, 19–21, 24–26, 37).

Strains with the *SUPor-PM25* (also designated RR126) and RR97 constructions were obtained from P. Geyer's lab. *P*(*white*) is the *P*-element transformation vector *CaSpeR3* (48, 49). This vector carries a mini-*white* gene containing approximately 300 bp of 5′ and 630 bp of 3′ flanking DNA, while a major portion of the first intron is deleted (42).

Small-scale *P*-element mobilization experiments were carried out as described elsewhere (48). The number of insertion sites was determined by Southern blot analysis designed to identify the flanking restriction fragments. For further analysis, only single independent transpositions were selected. The *CaSpeR3* transposon was mobilized in the same way as *SUPor-P M25* (48).

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Combinations of mutations located on the X chromosome (X*) and constructions with the marker *white* gene on an autosome were obtained according to the following scheme: ♀ X*/FM4 × ♂ *P{white}*/*P{white}*(autosome) or ♂*P{white}*−2/+; *P{white}*−3/+, where −2 and −3 denote the second and third chromosomes.

To combine the ph^{P1} mutation (1-0.5) with the $e(y)1^{P1}$ mutation, *y* ph^{P1} females were crossed to *f* $e(y)1^{P1}$ males. In F_1, *y* ph^{P1}/*f* $e(y)1^{P1}$ females were crossed to *y f* Bx^2 males. In F_2, *y* ph^{P1} f $e(y)1^{P1}$/*y f* Bx^2 females were selected and mated to *FM4* males. As a result, the *y* ph^{P1} *f* $e(y)1^{P1}$/*FM4* strain was obtained.

Compound strains with $su(Hw)^2$ and $su(Hw)^v$ mutations were obtained as described elsewhere (18a).

Eye color analysis was performed under a dissecting microscope with 3-day-old flies developing at 25°C. In each case, from 50 to 100 flies were scored to determine the eye color phenotype. Eye pigmentation was evaluated on the basis of pigmentation of the major part of its area. Analysis of pigmentation of flies with different allelic combinations was done as described previously (4, 5).

Preparation of the $P\{w^+, e(y)1^+\}$ and $P\{w^+, \Delta e(y)1\}$ constructions and *P*-element-mediated transformation. $P\{w^+, e(y)1^+\}$ was created by insertion of the *Hin*dIII-*Xho*I region of *e(y)1* into the *CaSpeR3* vector. $P\{w^+, \Delta e(y)1\}$ is P$\{w^+, e(y)1^+\}$ in which 80 nucleotides of *e(y)1* corresponding to amino acids 255 to 278 (GGAGGAGGATCATCTGGCGTTGGAGTGGCCGTCAAGCGGGAACG TGAGGAGGAGGAGTTTGAGTTTGTGACCAACTAGCG) were replaced by 91 nucleotides of the *Stalker* long terminal repeat (LTR) starting from the 3′-terminal nucleotide of *Stalker* and followed by 6 nucleotides of the *Eco*RI site (TGTAATAGATGTAATAGATTTGCTTTCCGAGCTCAGAACCTCTGCT CTGTTTGAATCTCTTTATTCGAATGATCAAAGTGTGCTGAAGTTGGA ATTC).

The $P\{w^+, e(y)1^+\}$ or $P\{w^+, \Delta e(y)1\}$ construct and p25.7wc (34) were injected into *y ac* w^{67c} preblastoderm embryos as described previously (50, 55). Chromosomal insertion of $P\{w^+, e(y)1^+\}$ or $P\{w^+, \Delta e(y)1\}$ was tested by the reversion of the white phenotype, and the number of copies was determined by Southern blot analysis using *P*-element sequences as a probe.

Construction of libraries. The cDNA library was constructed in the Uni-ZAP XR vector (Stratagene). The genomic library was constructed by cloning of DNA partially digested with endonuclease *Sau*3A in the λGEM11 vector. DNA and mRNA for the libraries were prepared from Oregon R adult flies.

RNA isolation and Northern blot analysis. Total cellular RNA was isolated from *Drosophila* embryos, larvae, pupae, or adult flies as described elsewhere (39). Poly(A)$^+$ RNA was selected on oligo(dT)-cellulose columns, and 1.5 μg of poly(A)$^+$ RNA was loaded per lane of agarose gel. After electrophoresis, the RNA was transferred to Hybond-N membranes (Amersham). Hybridization was performed at 50°C in high-SDS–formamide buffer (7% sodium dodecyl sulfate [SDS], 50% formamide, 5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 2% blocking reagent [Boehringer Mannheim], 50 mM sodium phosphate [pH 7.0], 0.1% sarcosyl) overnight. ^{32}P-labeled DNA probes were obtained in a random priming reaction. The membranes were washed two times in 0.1% SDS–1× SSC at room temperature for 10 min and for 20 min in 0.1% SDS–0.2× SSC at 65°C and then exposed to Kodak BioMax MS film with a Kodak BioMax MS intensifying screen for 2 to 4 h. Precise quantitation of the RNA in bands was done with a PhosphoImager (for Fig. 2) or with a photodensitometer (for Fig. 3).

3′-RACE of $e(y)1^{u1}$ mRNA. For 3′-RACE (rapid amplification of 3′ cDNA ends), the first cDNA strand was synthesized by using 0.5 μg of mRNA from $e(y)1^{u1}$ males with the $(GA)_{10}$ACTAGTCTCGAG$(T)_{18}$ primer and Superscript II reverse transcriptase (GibcoBRL). The product was purified in an agarose gel, and a two-step PCR was performed. For the first step, the following primers were used: GAGAGAGAGAACTAGTCTCGA and ATCCTGAAGGAGCTGAATG [sequences from the first exon of the *e(y)1* gene (Fig. 2)]. Then, a nested PCR with the same first primer and with the nested second primer CGTGGTCAAC CAACTGCT was performed (Fig. 2).

Protein expression and Western blot analysis. The pQE-30 expression vector (Qiagen) and *Escherichia coli* XL1-Blue were used for His-tagged production of e(y)1 and e(y)1^{u1} proteins. Affinity-purified rabbit polyclonal antibodies against the His-tagged e(y)1 protein were used in immunoprecipitation, Western blot analysis, and immunodetection experiments. These antibodies were tested to give signals of the same rate on Western blots with the wild-type and mutant proteins.

Protein extracts were obtained from nuclei isolated from adult flies as described elsewhere (6) and lysed in a buffer containing 50 mM Tris HCl (pH 8.8), 150 mM NaCl, 0.1% SDS, 1% Nonidet P-40, 0.5% sodium deoxycholate, aprotinin (0.02 mg/ml), and leupeptin (0.1 mg/ml). Immunoprecipitation was performed as described elsewhere (51); the protein samples were subjected to electrophoresis in SDS–10% polyacrylamide gels (SDS-PAGE) and electroblotted to nitrocellulose membranes (Amersham). Western blotting was performed with an enhanced chemiluminescence system (Amersham) according to the manufacturer's recommendations.

In situ hybridization to polytene chromosomes. *Drosophila* polytene chromosome spreads were prepared from salivary glands of the third-instar larvae grown at 17°C. Preparation of spreads, fixation, denaturation, and hybridization were done as described in reference 16. Labeling was performed with [α-^{3}H]dATP and [α-^{3}H]dUTP in a random priming reaction.

Immunostaining of polytene chromosomes. Fixation and squashing of salivary glands and antibody staining were performed as originally described by Platero et al. (45). Antibodies to $TAF_{II}40$ were used at 1:10 dilution. Cy3-conjugated anti-rabbit antibodies (1:300; Sigma) were used as secondary antibodies.

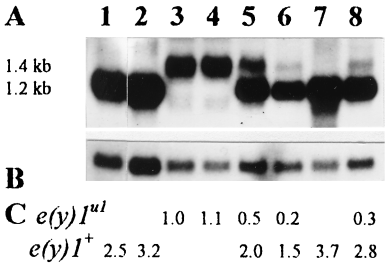

FIG. 1. Transcription of the $e(y)1^{u1}$ and $e(y)1^+$ genes. (A) Northern blot hybridization of the fragment of $e(y)1/TAF_{II}40$ cDNA (Fig. 2A) with mRNA from Oregon R males (lane 1), Oregon R embryos (lane 2), $e(y)1^{u1}/Y$ males (lane 3), $e(y)1^{u1}/e(y)1^{u1}$ females (lane 4), $e(y)1^{u1}/e(y)1^+$ females (lane 5), embryos from the ♀ $e(y)1^{u1}/e(y)1^+$ × ♂ $e(y)1^{u1}/Y$ cross (lane 6), $C(1)RM,_{yf}$ females (lane 7), and embryos from the ♀ $C(1)RM,_{yf}$ × ♂ $e(y)1^{u1}/Y$ cross (lane 8). (B) The same blot hybridized with the Ras2 probe. (C) Relative level of $e(y)1/TAF_{II}40$ transcription. The Northern blot was analyzed on a PhosphoImager; signals were normalized according to the results of Ras2 hybridization. The level of transcription in $e(y)1^{u1}/Y$ males was taken as 1.

In situ hybridization of tissue sections. The flies were fixed in Carnoy's solution for 1 h at room temperature. Paraffin embedding of the material and preparation of 7-μm sections were performed according to standard procedures (3). Digoxigenin (DIG) labeling of sense and antisense RNA and hybridization were performed according to the protocols for detection of mRNA with DIG-labeled RNA probes (Boehringer Mannheim).

Immunostaining of tissue sections. Paraffin embedding, fixation, and sectioning were performed as described for in situ hybridization. Incubation with primary antibodies was performed as described for immunostaining of polytene chromosomes. Secondary horseradish peroxidase-conjugated anti-rabbit antibodies (1:1,000; Amersham) and diaminobenzidine (DAB) staining were used for visualization. The sections were counterstained with fast green.

RESULTS

The *e(y)1* gene encodes the $TAF_{II}40$ protein. The $e(y)1^{u1}$ mutation was induced by the insertion of the *Stalker* mobile element (19). *Stalker* is present in more than 50 copies in most *D. melanogaster* strains (20). Therefore, we have developed a special strategy based on preparing two sets of strains with the same genetic background differing in the location of a single *Stalker* copy responsible for the $e(y)1^{u1}$ mutation (54). A clone containing *Stalker* and a flanking sequence of genomic DNA was obtained. The latter was used as a probe for screening the wild-type Oregon R library.

The 1.2-kb mRNA transcript changed in the $e(y)1^{u1}$ strain (Fig. 1) was detected by Northern blot hybridization. A cDNA clone was obtained and sequenced. The result of a BLAST (1) search indicated that this sequence was identical to that of the gene encoding the $TAF_{II}40$ protein (28).

To prove that the cloned gene was *e(y)1*, the genomic region of $TAF_{II}40$ localization (Fig. 2A) was inserted into the *CaSpeR3* vector and microinjected into embryos of the *C(1)RM,yf/y*^{2}*w e(y)*1^{u1}*/Y* strain. A complete reconstitution of the wild-type phenotype took place in five independent transgenic *y*^{2}*w e(y)*1^{u1} P$\{w^+, e(y)1^+\}$ lines of flies (Table 1), confirming that the cloned gene was indeed *e(y)1*. Thus, the *e(y)1* gene encodes the $TAF_{II}40$ protein.

Expression of the $e(y)1/TAF_{II}40$ gene during development. Northern blot hybridization was performed with mRNA isolated from the Oregon R strain at different developmental stages (Fig. 3). The transcription of the *e(y)1* gene appeared to be stage dependent. An increased level of transcription was detected at the pupal and embryonic stages, but the highest level of $e(y)1/TAF_{II}40$ mRNA—about five times higher than in adult males—was detected in adult females.

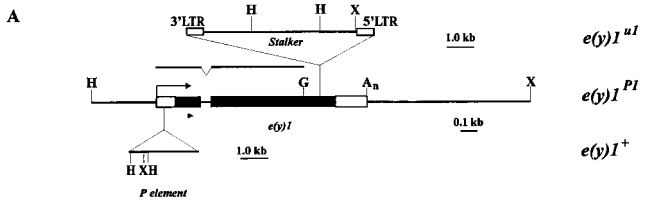

FIG. 2. Structure of the *e(y)1/TAF$_{II}$40* gene. (A) Map of the *e(y)1^{u1}* mutation. Black boxes, the coding regions of *e(y)1*; open boxes, transcribed, nontranslated regions. The arrow indicates the direction of transcription. H, *Hin*dIII; X, *Xho*I; G, *Bgl*II. The region shown was used for wild-type phenotype rescue. The upper line indicates the region from the cDNA clone, which was used as a probe in Northern blot hybridization. The position of primers for RACE is indicated by a triangle. (B) Amino acid sequence of the carboxy terminus of wild-type (upper line) and mutant (lower line) TAF$_{II}$40 protein.

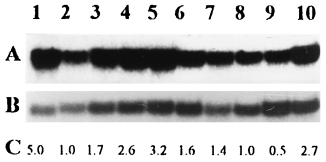

FIG. 3. Transcription of the *e(y)1/TAF$_{II}$40* gene at different stages of development of *D. melanogaster*. (A) Northern blot hybridization of a fragment of *e(y)1/TAF$_{II}$40* cDNA (Fig. 2A) with mRNA from the Oregon R strain. Samples are from adult females (lane 1) and males (lane 2); late (lane 3), middle (lane 4), and early (lane 5) pupae; late third (lane 6)-, early third (lane 7)-, second (lane 8)-, and first (lane 9)-instar larvae; and embryos (lane 10). (B) The same blot, hybridized with the Ras2 probe. (C) Relative level of *e(y)1/TAF$_{II}$40* transcription. Signals were normalized according to the results of Ras2 hybridization. The level of *e(y)1* transcription in males was taken as 1.

In situ hybridization on tissue sections of adult females demonstrated that the *e(y)1/TAF$_{II}$40* gene was highly expressed in trophocytes, follicular cells of gonads, and oocytes (Fig. 4A and B). The level of expression in all other tissues was much lower and did not significantly differ between tissues. Immunostaining with antibodies to the TAF$_{II}$40 protein also showed a high content of the protein in oocytes (Fig. 4C and D). Thus, the high level of *e(y)1/TAF$_{II}$40* expression in ovaries explains the fivefold difference in the mRNA content between females and males.

Molecular nature of the *e(y)1^{u1}* mutation: structural change of TAF$_{II}$40. Sequencing of the genomic copy of the *e(y)1* gene showed that the latter consisted of two exons. The *Stalker* element in the *e(y)1^{u1}* mutation is inserted at the second exon in the direction opposite that of gene transcription (Fig. 2A). The insertion was located at a position corresponding to the 25th amino acid from the carboxy terminus of the protein (Fig. 2B). Therefore, the mutant e(y)1 protein can be assumed to represent a chimeric protein containing a foreign amino acid sequence at its carboxy terminus.

To check this, *e(y)1^{u1}* mRNA was studied. On Northern blots, it had an apparent size of ca. 1.4 kb, thus being about 0.2 kb longer than the wild-type mRNA (Fig. 1). The 3′ end of *e(y)1^{u1}* mRNA was cloned by reverse transcription-PCR with mRNA obtained from the mutant strain. Its sequence showed that *e(y)1* mRNA terminated at different closely spaced sites within the 3′ LTR of *Stalker*. The chimeric protein was expected to be 270 amino acids in length, considering the location of the terminating codon within the *Stalker* sequence in all mRNAs (Fig. 2B). Thus, in the *e(y)1^{u1}* strain, 25 carboxy-terminal amino acids of TAF$_{II}$40 are replaced by 17 amino acids encoded by *Stalker* sequence, and the change in the molecular mass of the protein should be 0.65 kDa.

On the other hand, the difference in molecular masses of normal and mutated proteins detected by Western blot analysis was 5 kDa (Fig. 5). This discrepancy can be explained by the anomalous mobility of TAF$_{II}$40 in SDS-PAGE, because

TABLE 1. Interactions between *e(y)1* constructions and *y^2*, *e(y)1^{u1}*, and *e(y)3^{u1}* mutations[a]

Genotype	Pigmentation of bristles[b]				Survival[c]
	Th	L	W	Ab	
y^2 e(y)1^{u1}	1	2	5	5	ND
y^2 e(y)1^{u1};P{e(y)1$^+$}-1-5/+	5	5	5	5	ND
y^2 e(y)1^{u1};P{Δe(y)1}-1/+	2	3	5	5	ND
y^2 e(y)1^{u1};P{Δe(y)1}-2/+	3	3	5	5	ND
y^2 e(y)1^{u1};P{Δe(y)1}-2/*P{Δe(y)1}*-2	4	5	5	5	ND
y^2 e(y)1^{u1};P{Δe(y)1}-3/+	4	4	5	5	ND
y^2 e(y)1^{u1};P{Δe(y)1}-2/*P{Δe(y)1}*-3	5	5	5	5	ND
y^2 e(y)1^{u1};P{Δe(y)1}-4/+	3	4	5	5	ND
y^2 e(y)1^{u1};P{Δe(y)1}-5/+	3	4	5	5	ND
y^{2}w e(y)3^{u1}	3	3	5	5	54
y^{2}w e(y)1^{u1}e(y)3^{u1}					0
y^{2}w e(y)1^{u1}e(y)3^{u1};P{e(y)1$^+$}-1-5/+	3	4	5	5	42–51
y^{2}w e(y)1^{u1}e(y)3^{u1};P{Δe(y)1}-1/+	1	1	1	2	7
y^{2}w e(y)1^{u1}e(y)3^{u1};P{Δe(y)1}-2/+	0	1	2	2	15
y^{2}w e(y)1^{u1}e(y)3^{u1};P{Δe(y)1}-3/+	1	2	2	3	11
y^{2}w e(y)1^{u1}e(y)3^{u1};P{Δe(y)1}-4/+	1	2	2	3	11
y^{2}w e(y)1^{u1}e(y)3^{u1};P{Δe(y)1}-2/*P{Δe(y)1}*-3	2	2	4	5	17

[a] Abbreviations: *P{e(y)1$^+$}*-1-5 or *P{Δe(y)1}*-1-5, different single insertions of *P{w$^+$, e(y)1$^+$}* or *P{w$^+$, Δe(y)1}* in five strains; *P{Δe(y)1}*-2, single insertion in the second chromosome; *P{Δe(y)1}*-3, single insertion in the third chromosome; Th, thoracal bristles; L, leg bristles; W, wing bristles; Ab, abdominal bristles.

[b] Level of pigmentation in 3- to 5-day-old males developing at 25°C, ranked on a scale of 0 (pigmentation of *y^1* flies) to 5 (pigmentation of *y$^+$* flies). Flies with well-characterized *y* alleles were used to define the control level of pigmentation (4, 5). The effects of constructions with the *e(y)1* gene on the *y^2 e(y)1^{u1}*, *y^2 e(y)3^{u1}*, and *y^2 e(y)1^{u1} e(y)3^{u1}* mutation combinations was studied in the crosses of *y^2 e(y)1^{u1}/FM4*, *y^2 e(y)3^{u1}/FM4*, and *y^2 e(y)1^{u1}e(y)3^{u1}/FM4* females with *y^{2}w e(y)1^{u1};P{Δe(y)1}/P{Δe(y)1}* and *y^{2}w e(y)1^{u1};P{e(y)1$^+$}/P{e(y)1$^+$}* males. *FM4* is an abbreviation for *FM4,y^{31d}sc^{8}B*, the balancer for the X chromosome.

[c] Percentage of surviving males with a given phenotype, calculated as a ratio between given males and *FM4* males. ND, not determined.

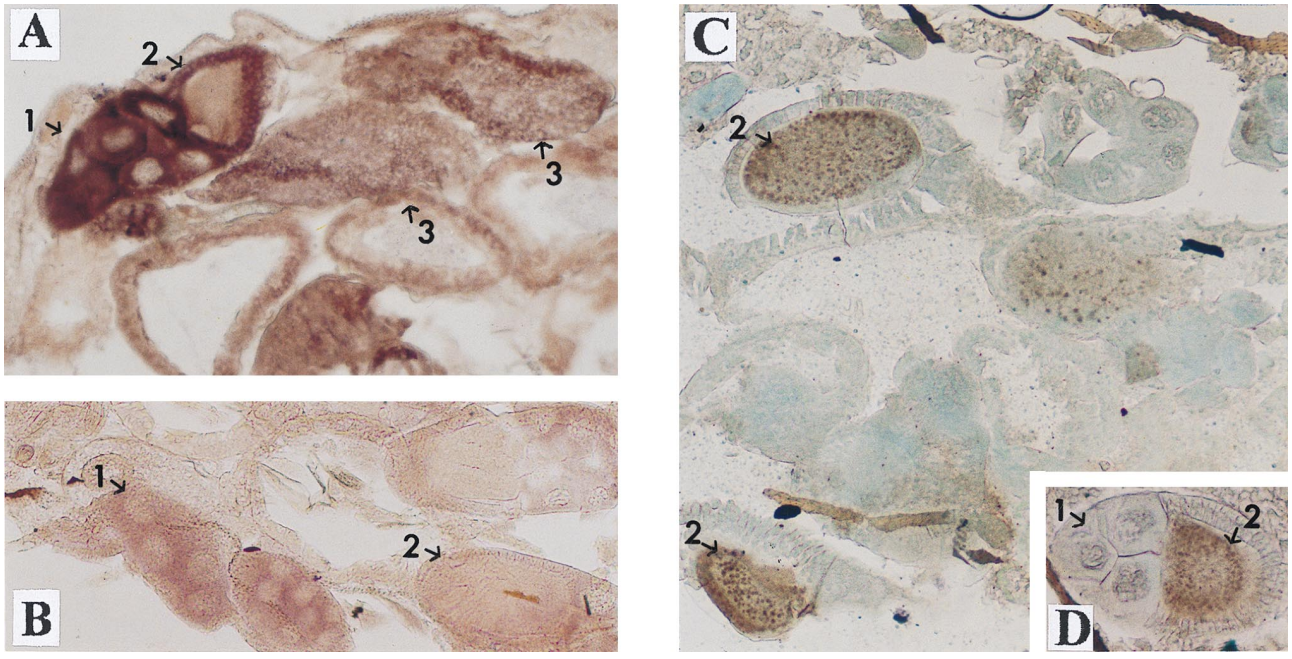

FIG. 4. Expression of *e(y)1* in different tissues of Oregon R flies. (A and B) In situ hybridization of a frontal tissue section of female abdomen with the DIG-labeled *e(y)1* antisense (A) and sense (B) RNA probes. (C and D) Immunostaining of a frontal tissue section of female abdomen with antibodies to e(y)1 protein. Horseradish peroxidase and DAB were used for visualization; the tissue was counterstained with fast green. One can see a high level of *e(y)1* transcription and expression in ovaries: 1, in trophocytes; 2, in primary oocytes; 3, in mature oocytes. Note that while the level of *e(y)1* mRNA content is high in trophocytes and mature oocytes and low in primary oocytes, the $TAF_{II}40$ protein is predominantly detected in oocytes rather than in trophocytes (C). Magnification, ×130.

the wild-type and mutated proteins synthesized in the bacterial system had a similar difference in molecular mass (data not shown). Six glutamic amino acids were deleted in the $e(y)1^{u1}$ protein, which may have greatly affected the mobility of the protein.

Loss of the carboxy terminus does not affect the ability of $TAF_{II}40$ to bind to chromatin. $TAF_{II}40$ was detected in numerous sites on polytene chromosomes of the Oregon R strain (Fig. 6). The distribution of $TAF_{II}40$ on chromosomes of the $e(y)1^{u1}$ mutant was the same as on the wild-type chromosomes, although the content was decreased. However, the latter finding can be explained by a lower level of polytenization.

Inhibition of *e(y)1* transcription in the $e(y)1^{u1}$ flies. The insertion of *Stalker* also interferes with *e(y)1* transcription, possibly as a consequence of the activation of *Stalker* transcription from the 3′ LTR in the direction opposite that of the gene. A decrease of the *e(y)1* mRNA content in mutated flies was detected at all stages of development (Fig. 1 and 7). In adult flies, the content of $e(y)1^{+}$ mRNA was four times higher than that of $e(y)1^{u1}$ mRNA in heterozygous $e(y)1^{u1}/e(y)1^{+}$ females (Fig. 1, lane 5) and 2.5 times higher in $e(y)1^{+}$ males than in $e(y)1^{u1}$ males (Fig. 1, lanes 1 and 3).

The lowest ratio of $e(y)1^{u1}$ mRNA to $e(y)^{+}$ mRNA, equal to 1:9 to the progeny of the cross of *C(1)RM,yf* females to $e(y)1^{u1}/Y$ males, was found in embryos (Fig. 1, lane 8). This finding is not surprising, as the eggs were laid by females bearing only the wild-type copy of the gene and according to in situ hybridization, they should contain a large amount of maternal mRNA (see above). The presence of a weak 1.4-kb band (Fig. 1, lane 8) should represent $e(y)1^{u1}$ mRNA synthesized in embryos. A more interesting finding was that the ratio of $e(y)1^{u1}$ mRNA to $e(y)^{+}$ mRNA was almost equally low in embryos from the cross of $e(y)1^{u1}/e(y)1^{+}$ females to $e(y)1^{u1}/Y$ males (Fig. 1, lane 6), revealing either the absence or an extremely low content of $e(y)1^{u1}$ mRNA in the maternal mRNA of embryos. This means that homozygous $e(y)1^{u1}/e(y)^{u1}$ females may have difficulty supplying their oocytes with $e(y)1^{u1}$ mRNA. On the other hand, immunohistochemistry detected the presence of the $TAF_{II}40$ protein in the residual ovaries of $e(y)1^{u1}/e(y)1^{u1}$ homozygous females (Fig. 8).

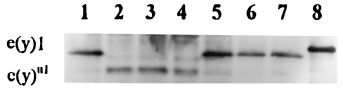

FIG. 5. Western blot analysis of *e(y)1* expression. Shown are results of immunoprecipitation of e(y)1 and $e(y)1^{u1}$ proteins from nuclear extracts from adult flies of the Oregon R (lane 1), $e(y)1^{u1}$ (lanes 2 to 4), and $e(y)1^{+}$ strains (lanes 5 to 7) and the recombinant His-tagged protein (lane 8). The positions of e(y)1 and $e(y)1^{u1}$ proteins are shown on the left.

The main biological effects of the $e(y)1^{u1}$ mutation depend on partial inhibition of *e(y)1* transcription. The $e(y)1^{u1}$ mutation did not affect the viability of flies, and no visible morphological changes were detectable in adult mutant flies. However, females homozygous for the $e(y)1^{u1}$ mutation were sterile. The ovaries of mutant flies were found to be dramatically underdeveloped. They were very small and did not contain mature oocytes (Fig. 8 and 9). Microinjection of a construction with the $e(y)1^{+}$ gene restored normal fertility and ovary morphology in homozygous $e(y)1^{u1}$ females, confirming the dependence of ovary development on the e(y)1 phenotype.

There may be two possible explanations for female sterility. One is that the normal level of *e(y)1* expression is important for oocyte development; the second is that the unchanged carboxy terminus of $TAF_{II}40$ is essential for the expression of some genes involved in the maturation of oocytes. To test these two possibilities, we made an attempt to rescue the wild-type phenotype by microinjection of the $P\{w^{+}, \Delta e(y)1\}$ construction,

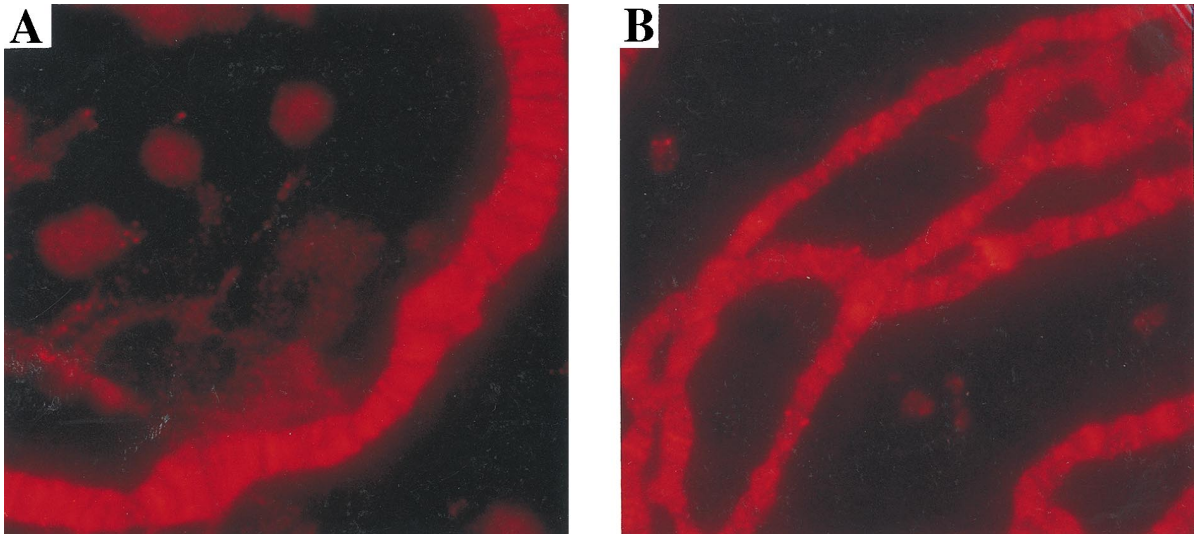

FIG. 6. Immunostaining of polytene chromosomes from wild-type Oregon R (A) and *e(y)1^{u1}* (B) larvae with antibodies against e(y)1 and Cy3-conjugated secondary antibodies. Original magnification, ×1,000.

which expressed exactly the same mutant TAF$_{II}$40 protein with *Stalker* amino acids at the end as *e(y)1^{u1}* flies (Fig. 2). Five w^+ revertants bearing the construction in different sites of autosomes were obtained. In all cases, the fertility of *e(y)1^{u1}* females was restored. Thus, it is the reduced transcription of the *e(y)1* gene that leads to the sterility of *e(y)1^{u1}* females.

TAF$_{II}$40 is indispensable. We have also obtained another mutation of the *e(y)1* gene induced by insertion of the *P* element. This allele was isolated in *P-M* hybrid expression dysgenesis as the *e(y)4^{P1}* mutation and had a milder phenotype in comparison to *e(y)1^{u1}*: males had shortened and thin bristles, while females were morphologically normal and fertile. The mutation was genetically localized in approximately the same region of the X chromosome as *e(y)1^{u1}* (19). Southern blot analysis showed that insertion of the *P* element occurred in the *e(y)1* gene. The exact site of the insertion was cloned by PCR using the *P*-element and *e(y)1* sequences as primers (Fig. 2). As it is located in the *e(y)1* gene, the designation *e(y)1^{P1}* will be used hereafter.

The *e(y)1^{P1}* mutation is induced by insertion of the *P* element into the transcribed noncoding 5′ region of the gene (Fig. 2). Thus, the coding region of the gene is not damaged, and we also did not detect any changes in the level of *e(y)1* transcription by Northern blot hybridization (data not shown).

Recently we have developed a method to dramatically increase the effect of the *P* element on transcription of a target gene by introducing the *ph^{P1}* mutation (5). The latter was induced by *P*-element insertion in the *polyhomeotic* (*ph*) gene, resulting in expression of the chimeric P-Ph protein consisting of the DNA-binding domain of *P*-element transposase and an almost complete Ph protein sequence. The P-Ph protein binds the *P*-element sequences and recruits to this site other members of the Pc-repressive complex. This leads to blocking of transcription from promoters located in close vicinity to the *P*-element insertion (5).

The combination of the *e(y)1^{P1}* mutation with *ph^{P1}* led to the lethal phenotype. The wild-type phenotype could be restored by transformation of *e(y)1^{P1} ph^{P1}* flies with the P{w^+, *e(y)1$^+$*} construction. To detect the stage of death, we crossed *e(y)1^{P1} ph^{P1}/FM4* females to *e(y)1^{P1}* males. We found that embryos died at the middle and late embryonic stages (from stages 9 to 14). Thus, the TAF$_{II}$40 protein is indispensable.

The *e(y)1^{u1}* mutation inhibits *yellow* expression in bristles but not in the body and wings. The *e(y)1^{u1}* allele does not change the viability and phenotype of flies, suggesting that the transcription of most genes is not sensitive to a moderate decrease in the concentration of truncated TAF$_{II}$40. However, the *e(y)1^{u1}* mutation was shown to inhibit the expression of several genes in the case of their partial inactivation as a result of insertion of foreign sequences, partial deletion of enhancer, or a mutation in *trans*-regulatory gene (17, 19, 21). These events probably make transcription more sensitive to the influence of *e(y)1^{u1}*.

The *yellow* and *white* genes were further used to study some features of TAF$_{II}$40 activity. The *yellow* gene contains different enhancers responsible for *yellow* expression in the wings, body, and bristles (22). The question was whether *yellow* expression driven by different enhancers was equally sensitive to *e(y)1^{u1}*. The previously used *y^2* mutation was not suitable to clarify this question as the body and wing enhancers were blocked by an insulator, *gypsy* su(Hw)-binding region (27). Therefore, we checked the effect of the *e(y)1^{u1}* mutation on some other *yellow* alleles (Fig. 10).

The *e(y)1^{u1}* mutation interfered with *yellow* expression in revertants of *y^2* flies associated with rearrangements of *gypsy* but failed to affect the pigmentation of revertants which lacked the whole *gypsy* insertion except one LTR (*y^{+1MC}*, *y^{+3MC}*). The *e(y)1^{u1}* mutation had the strongest effect in combination with

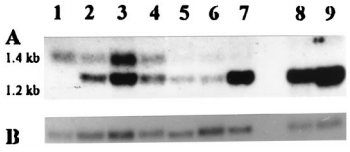

FIG. 7. Effect of the *e(y)1^{u1}* mutation on *e(y)1* transcription. (A) Northern blot hybridization of the fragment of *e(y)1/TAF$_{II}$40* cDNA (Fig. 2A) with mRNA isolated at different stages of development of the progeny of the ♀ *C(1)RM, yf* × ♂ *e(y)1^{u1}/Y* cross: males (lane 1); late (lane 2), middle (lane 3), and early (lane 4) pupae; third (lane 5)- and first (lane 6)-instar larvae; embryos (lane 7); and adult females (lane 8). Lane 9, mRNA from females of the Oregon R strain. (B) The same blot, hybridized with the Ras2 probe.

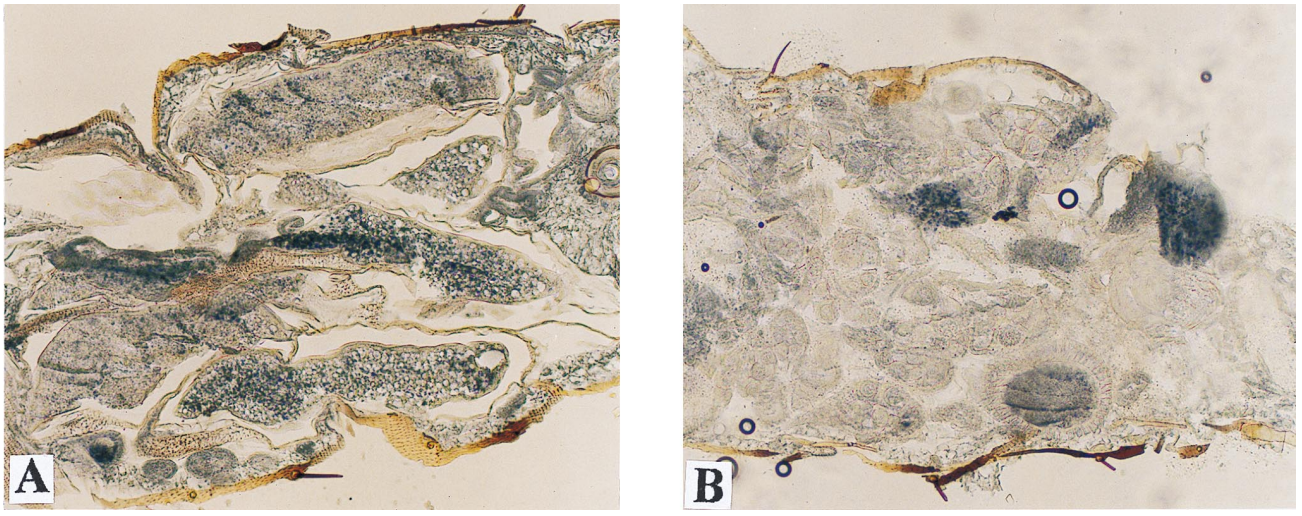

FIG. 8. Ovaries from wild-type (A) and *e(y)1*^{u1} (B) flies. Immunostaining of frontal tissue section of female abdomen with antibodies to the e(y)1 protein. Horseradish peroxidase and Sigma fast DAB with a metal enhancer were used for visualization. Magnification, ×70.

the y^{+2MC} revertant, which was induced by the insertion of *jockey* and a deletion of the su(Hw)-binding region of *gypsy* (17, 25). $y^{+2MC}e(y)1^{u1}$ flies had the same color of the bristles as flies lacking the bristle enhancer, while the body and wings remained normally pigmented (Fig. 10). Similar results were obtained in experiments with two partial y^{2} revertants (24) which had the su(Hw)-binding region disrupted by the insertion of either *jockey* (y^{2PR1}) or *hobo* (y^{2PR2}). Flies from both

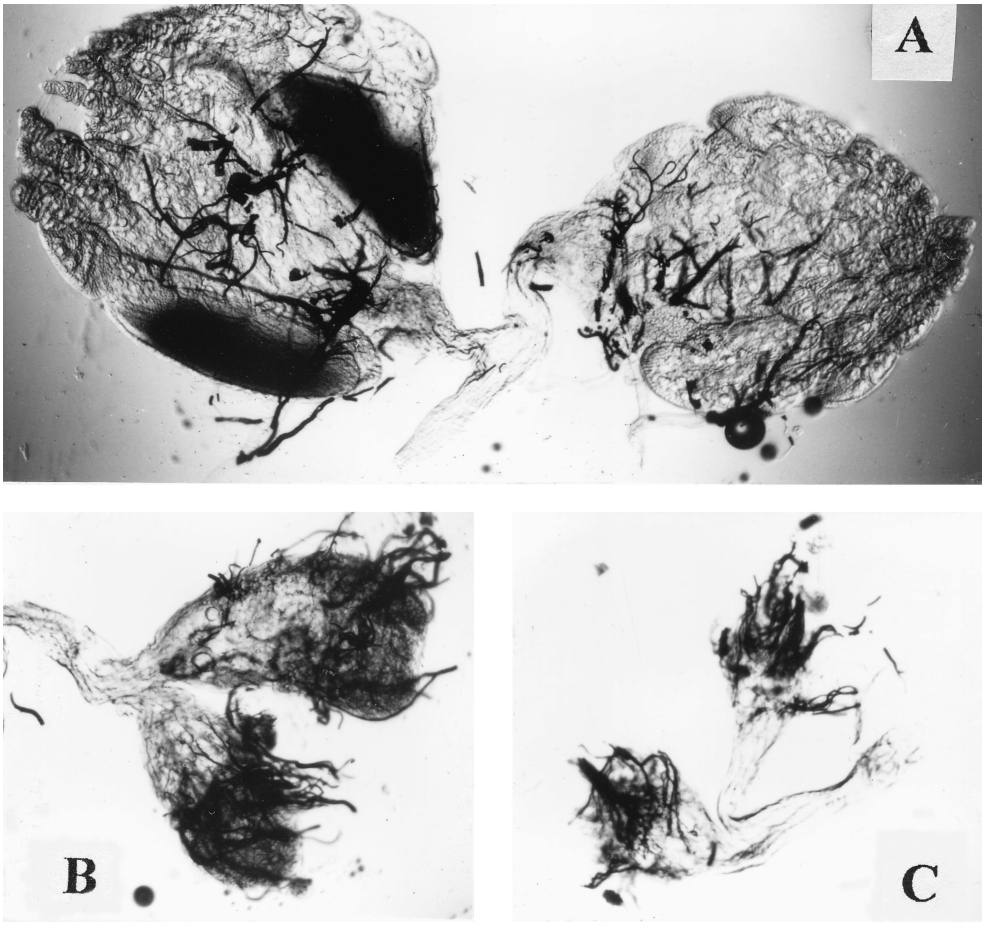

FIG. 9. Ovaries from wild-type (A) and *e(y)1*^{u1} (B and C) flies (total preparation). Magnification, ×40.

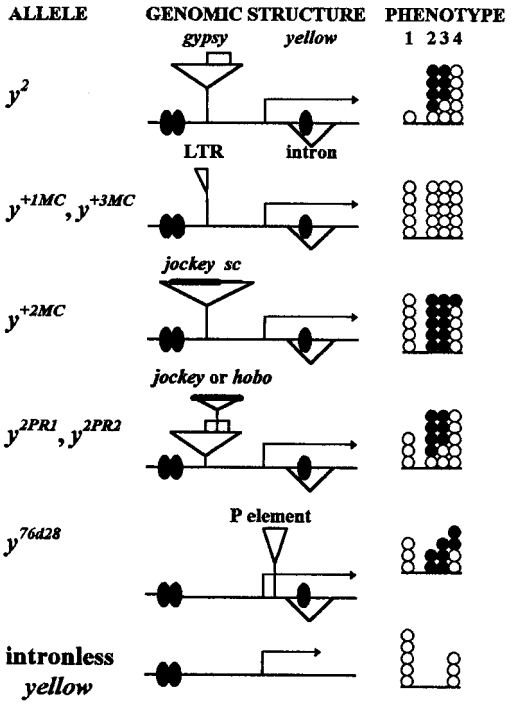

FIG. 10. Genetic analysis of interaction of *y* alleles with *e(y)1^{u1}* mutation. The schemes for *y* alleles are not to scale. *yellow* transcripts are shown by arrows; transcriptional enhancers are indicated by shaded ovals. The enhancers that control *yellow* expression in the wings and body cuticle are located in the 5′-upstream region of the *yellow* gene, whereas enhancers controlling *yellow* expression in the bristles reside in the intron of the gene (22). The su(Hw)-binding region is indicated by empty boxes; insertions found in the various alleles are represented by triangles. The total number of circles in the phenotype column indicates the levels of pigmentation of the body and wings (column 1), thoracic bristles (column 2), leg bristles (column 3), and abdominal bristles (column 4). The number of black circles shows the inhibitory effect of the *e(y)1^{u1}* mutation on *yellow* expression for different *y* alleles. Each circle represents one point on the scale described in the footnote to Table 1.

strains have an intermediate coloration of the body and wings which was very sensitive to any modification of the transcription level. Again the *e(y)1^{u1}* mutation reduced the pigmentation of bristles in the same way as in the original *y^2* allele, but it did not change the level of body and wing pigmentation (Fig. 10).

We also tested the *y^{76d28}* mutation caused by the insertion of the *P* element into the 5′-transcribed, untranslated portion of the *yellow* gene (26). The color of all adult cuticular structures is tan in *y^{76d28}* flies, indicating that *yellow* gene expression decreases in all cell types to a level intermediate between that of wild-type flies and that of flies carrying a deficiency for the *yellow* gene. As in the previous cases, the *e(y)1^{u1}* mutation reduced the pigmentation of bristles but not of the body and wings in *y^{76d28}* flies.

Role of carboxy termini in TAF$_{II}$40 protein function. As shown above, the *P*{*w$^+$, Δe(y)1*} construction expressing the truncated version of TAF$_{II}$40 protein restores the fertility of *e(y)1^{u1}* females. However, fertility is a qualitative factor that does not allow for quantitative assessment of the role of the carboxy terminus in the TAF$_{II}$40 function. Thus, it would be interesting to compare the effects of *P*{*w$^+$, Δe(y)1*} and *P*{*w$^+$, e(y)1$^+$*} constructions on the bristle pigmentation of *y^2 w e(y)1^{u1}* flies. Five strains with a single *P*{*w$^+$, Δe(y)1*} construction and five strains with a single *P*{*w$^+$, e(y)1$^+$*} construction located on the second or third chromosome possessing orange or dark orange eyes were selected. The levels of *e(y)1* expression in the constructions were comparable, as shown by Northern blot analysis of *y^2 w; P*{*w$^+$, Δe(y)1*}, and *y^2 w e(y)1^{u1};P*{*w$^+$, e(y)1$^+$*} males (not shown).

All five tested *P*{*w$^+$, Δe(y)1*} constructions in heterozygote (a single copy of the construction per genome) only partially restored the bristle pigmentation of *y^2 w e(y)1^{u1}* flies. On the other hand, a single copy of any of five *P*{*w$^+$, e(y)1$^+$*} constructions completely suppressed the mutant phenotype of the *e(y)1^{u1}* allele (Table 1). Combination of two different *P*{*w$^+$, Δe(y)1*} constructions in heterozygote or in homozygote [two copies of the same *P*{*w$^+$, Δe(y)1*} construction] led to a stronger suppression of mutant bristle phenotype. This result suggests that more truncated protein is required for restoring the *yellow* expression in bristles.

Similar results were obtained in experiments with the *e(y)1^{u1} e(y)3^{u1}* combination of mutations. By itself, the *e(y)3^{u1}* mutation only mildly decreased the viability of flies. However, the combination of the *e(y)1^{u1}* mutation with *e(y)3^{u1}* is lethal at the late larval and early pupal stages of development (21). The viability and bristle pigmentation of flies carrying the *e(y)1^{u1} e(y)3^{u1}* combination were completely restored in three independent strains with the *P*{*w$^+$, e(y)1$^+$*} construction. The *P*{*w$^+$, Δe(y)1*} constructions only partially rescued the viability of *e(y)1^{u1} e(y)3^{u1}* flies (Table 1).

Similarly, the surviving *y^2 e(y)1^{u1} e(y)3^{u1};P*{*w$^+$, Δe(y)1*}/+ flies still had a strong mutant bristle phenotype. Combination of two different *P*{*w$^+$, Δe(y)1*} constructions in heterozygote led to more prominent suppression of mutant phenotype.

Effect of the *e(y)1^{u1}* mutation on *white* expression. It was found previously that the *e(y)1^{u1}* mutation suppressed the enhancer-dependent transcription of the *white* gene in the absence of the zeste protein (21). The *white* gene has an enhancer element located in the 5′-upstream region (36, 44, 60). In the absence of the upstream enhancer, the eyes are yellow. The combination of *z^{v77h}* and *e(y)1^{u1}* mutations, each of which does not significantly affect *white* expression, strongly and synergistically decreases the eye pigmentation almost to the level typical of enhancerless flies (21).

To further study the role of the *e(y)1^{u1}* mutation in activation of the *white* promoter by enhancers, we used the *y$^-$ac$^-$w^{1118}* strain with a mini-*white CaSpeR3* construction which contained a mini-*white* gene without an eye enhancer (42). In general, *y$^-$ac$^-$w^{1118} CaSpeR3* flies have yellow eyes, the residual color being maintained by the promoter-dependent transcription. The mini-*white* construction was mobilized by crosses with the Δ2-3(99B) strain, and 17 strains with a single insertion of the mini-*white* construction on the second or third chromosome that possessed eye color from dark orange to red were selected (Table 2). The activation of *white* expression in enhancerless constructions may be explained by the presence of a foreign enhancer element in the neighborhood of the *white* gene and by a local structure of chromatin. In 12 of 17 strains, the *e(y)1^{u1}* mutation strongly or moderately reduced the level of eye pigmentation (Tabel 2). This result suggests that *white* expression is sensitive to the *e(y)1^{u1}* mutation.

Insulation by the su(Hw)-binding region makes *white* transcription insensitive to the *e(y)1^{u1}* mutation. The *SUPor-P* construction contains the mini-*white* gene and its eye enhancer framed by two su(Hw)-binding regions. The latter makes *white* transcription independent of the genomic position (48, 49). We obtained eight different strains carrying the *SUPor-P* construction in different sites of the second chromosome. All of them had the wild-type red-colored eyes (Table 2).

The combination of *SUPor-P* constructions with *e(y)1^{u1}* and

TABLE 2. Influence of the $e(y)1^{u1}$ mutation on *white* expression in different *white* constructions

Construction[a]	No. of strains	Genotype	Phenotype[b] (no. of strains)
P(*white*)	5	+	Red
		$e(y)1^{u1}$	Red (3), brown (1), yellow-orange (1)
	8	+	Brown
		$e(y)1^{u1}$	Brown (2), orange (2), yellow-orange (3), yellow (1)
	4	+	Orange
		$e(y)1^{u1}$	Yellow (4)
P(BR/Eye/*white*/BR) [*SUPor-P* transposon]	5	+	Red
		$su(Hw)^2/su(Hw)^v$	Red
		z^{v77h} $e(y)1^{u1}$	Red
		z^{v77h} $e(y)1^{u1}$;$su(Hw)^2/su(Hw)^{vv}$	Yellow-orange
	3	+	Red
		$su(Hw)^2/su(Hw)^v$	Brown-orange
		z^{v77h} $e(y)1^{u1}$	Red
		z^{v77h} $e(y)1^{u1}$;$su(Hw)^2/su(Hw)^{vv}$	Yellow
P(Eye/BR/*white*) [RR97 transposon]	3	+	Brown
		z^{v77h} $e(y)1^{u1}$	Yellow-orange

[a] P(BR/Eye/*white*/BR) (48, 49) has two su(Hw)-binding regions (BR) flanking the eye enhancer (Eye) and the CaSpeR mini-*white* gene. P(Eye/BR/*white*) (49) contains the su(Hw)-binding region inserted between the eye enhancer (Eye) and the CaSpeR mini-*white* gene.

[b] A low level of *white* expression produces the yellow eye phenotype, whereas wild-type expression gives the red eye color. The number of strains showing the specified eye color is given in parentheses.

z^{v77h} mutations did not influence eye color. On the other hand, an additional introduction of $su(Hw)^2/su(Hw)^v$ mutations inactivating the *su(Hw)* gene (29, 41) led to the inhibition of *white* expression in the presence of $e(y)1^{u1}$ and z^{v77h} mutations (Table 2). In three cases, $su(Hw)^2/su(Hw)^v$ mutations alone induced a slight inhibition of *white* expression, but it was much weaker.

To test the possibility that the su(Hw) protein itself can activate *white* expression in the presence of $e(y)1^{u1}$ and z^{v77h}, we used the RR97 construction, obtained from P. Geyer, where the su(Hw)-binding region was inserted between the eye enhancer and the *white* promoter (49). Flies from three independent strains with a single insertion of RR97 had brown eyes. The introduction of $e(y)1^{u1}$ and z^{v77h} mutations enhanced the mutant *white* phenotype (Table 2), indicating that the su(Hw) protein could not directly activate *white* expression in the $e(y)1^{u1}$ z^{v77h} combination of alleles.

DISCUSSION

The *e(y)1* gene encodes a $TAF_{II}40$ protein. The main result obtained is that one of abundant TAFs, i.e., $dTAF_{II}40$, is encoded by the previously described *e(y)1* gene. On the basis of some genetic data, the latter was suggested to be involved in the control of long-distance interactions, in particular between *yellow* and *white* enhancers and promoters (19, 21).

$TAF_{II}40$ is incorporated into the TFIID multiprotein complex (47, 59). It has been proposed that various classes of gene-specific activators interact with one or more TAFs in order to provide transcription of their target genes. In vitro protein-protein interaction assay revealed a direct binding of $dTAF_{II}40$ or its human homologue $hTAF_{II}31$ with an activation domain of several transcription factors (28, 35, 38, 57). It has been postulated that the $TAF_{II}40$ protein mediates the activation by proteins with acidic domains. Thus, $TAF_{II}40$ possesses the features that can be expected for the protein product of the *e(y)1* gene.

We have found that the wild-type $TAF_{II}40$ protein seems to be involved in the organization of transcription from a large group of promoters, as it is present in practically every band of a polytene chromosome. In addition, $TAF_{II}40$ expression was detected in all organs of adult flies. An elevated level of expression was detected at the embryonic and pupal stages of development, when the growth of new tissues is prominent.

A particularly high level of $TAF_{II}40/e(y)1$ expression was found in female gonads, which leads to an approximately fivefold difference in the *e(y)1* mRNA content between females and males. As a result, large amounts of mRNA and the protein accumulate in oocytes. This finding indicates that the $TAF_{II}40/e(y)1$ gene may be a maternal gene and suggests an important role of the $TAF_{II}40$ protein in gene activation during early embryogenesis.

In vivo consequences of *e(y)1* mutations. Here we have described for the first time mutations of the gene encoding the $TAF_{II}40$ protein in higher eucaryotes. One of them, the $e(y)1^{P1}$ mutation, is induced by *P*-element insertion and has almost no influence on *e(y)1* expression. However, its effect can be significantly enhanced in combination with the ph^{P1} mutation, known to repress transcription of genes with a *P*-element insertion in the neighborhood of the promoter element (5). The ph^{P1} $e(y)1^{P1}$ combination is lethal at the middle embryonic stage of development, indicating that $TAF_{II}40$ is an indispensable protein.

Survival of embryos throughout stages 9 to 14 can be explained by a high concentration of $TAF_{II}40$ in oocytes. Similar results were obtained for $TAF_{II}60$ and $TAF_{II}110$ (52). A large maternal contribution of wild-type $TAF_{II}60$ and $TAF_{II}110$ supported the first 15 to 16 stages of embryogenesis against the null-mutant background.

Another mutation, $e(y)1^{u1}$, is induced by the *Stalker* mobile element insertion into the coding sequence of the *e(y)1* gene. This insertion leads to two effects: (i) truncation of $TAF_{II}40$ with replacement of 25 carboxy-terminal amino acids by 17 foreign amino acids and (ii) a decrease of the level of *e(y)1* transcription. The mutation results in a dramatic underdevelopment of ovaries leading to female sterility and in a mild repression of transcription of several genes.

We found that female fertility could be restored by the synthesis of truncated $TAF_{II}40$ protein and demonstrated that this major effect of $e(y)1^{u1}$ mutation depended on reduced *e(y)1* transcription rather than on $TAF_{II}40$ structural changes. A specific effect on the development of ovaries may be ex-

plained either by a stronger inhibition of *e(y)1* transcription by *Stalker* in ovaries or by a selective sensitivity of the expression of some genes critical for ovary development.

TATA-less promoters are sensitive to weak mutation in the *TAF$_{II}$40/e(y)1* gene. It was found recently that the dTAF$_{II}$60-dTAF$_{II}$40 heterotetramer bound to the downstream promoter element (DPE), a distinct 7-nucleotide core promoter element located about 30 nucleotides downstream of the transcription start site of many TATA-box-deficient (TATA-less) promoters in *Drosophila* (9, 10). It was suggested that the dTAF$_{II}$60-dTAF$_{II}$40 heterotetramer plays a direct role in basal transcription of TATA-less DPE-containing genes.

Expression of the *white* gene was found to be sensitive to the combination of z^{v77h} and $e(y)1^{u1}$ mutations (21). Here we have demonstrated that *white* expression is frequently and strongly influenced by the $e(y)1^{u1}$ mutation alone in enhancerless constructions putatively activated by different foreign enhancers. On the other hand, it is known that the *white* gene contains a TATA-less promoter with a DPE core sequence. This agrees with a strong dependence of *white* expression on the TAF$_{II}$40 protein content.

Our data also demonstrate that the $e(y)1^{u1}$ mutation moderately reduced *yellow* expression in the bristles but not in the body cuticle and wing blades. The *yellow* gene has a typical TATA box. However, we recently found that deletion of the TATA promoter affected only body and wing pigmentation, not *yellow* expression in bristles in the presence of a strong enhancer element (18). This finding suggests the presence of an internal promoter element interacting with the bristle enhancer and activating *yellow* expression in bristles. The sequence of the putative *yellow* promoter region has no homology to the DPE-containing promoters (9, 10), but the canonic DPE sequence is present in only 20% of TATA-less promoters. Thus, in vivo TATA-less promoters represent a group of promoters that are most sensitive to the reduction of the TAF$_{II}$40 content.

A possible role of the TAF$_{II}$40 carboxy-terminal domain in vivo. As was shown, the mutant phenotype of the $e(y)1^{u1}$ allele could be at least partially reversed by supplying an additional amount of truncated e(y)1/TAF$_{II}$40 protein, in agreement with the results of in vitro experiments. It has been shown that 222 amino-terminal amino acids of TAF$_{II}$40 harbor domains for interactions with basic factors, activators, and other TAFs (28). dTAF$_{II}$40 and hTAF$_{II}$31 have significant homology only in their amino termini (28). The carboxy-terminal portion of dTAF$_{II}$40 bears similarity to many glycine-rich proteins (28), but in vitro experiments reveal no function of the carboxy terminus in protein-protein interactions.

However, any tested single copy of the $P\{w^+, \Delta e(y)1\}$ construction does not completely compensate for the effect of the $e(y)1^{u1}$ mutation on the y^2 phenotype or the lethal phenotype of the $e(y)1^{u1}\ e(y)3^{u1}$ combination of mutations. Even the presence of two doses of the $P\{w^+, \Delta e(y)1\}$ construction fails to completely rescue the y^+ phenotype or suppress the lethal phenotype of the $e(y)1^{u1}\ e(y)3^{u1}$ combination of mutations. On the other hand, a single dose of the $P\{w^+, e(y)1^+\}$ construction has a much stronger suppression effect.

Thus, deletion of the carboxy-terminal amino acids seems to make expression of tagged genes more sensitive to the concentration of TAF$_{II}$40 protein. We speculate that the carboxy-terminal portion of TAF$_{II}$40 promotes an effective binding of the protein to the DNA covered by nucleosomes. This may explain the compensation of its loss by an increase of the mutant protein concentration. It is worth noting that the deleted carboxy-terminal part of TAF$_{II}$40 contains the only charged stretch of the protein (total charge is −6). As human TAF$_{II}$31 also has a single charged stretch (total charge is −13) located at its carboxy terminus, this similarity may reflect some special function of this region. The negatively charged carboxy terminus is a characteristic feature of many transcription factors as well as the HMG-1 and HMG-2 families (12).

It is not clear why expression of the *white* gene flanked by the su(Hw)-binding regions is independent of the combination of the $e(y)1^{u1}$ and z^{v77h} mutations. The $e(y)1^{u1}$ mutation in combination with the z^{v77h}-null allele strongly reduces *white* expression (21). However, two su(Hw)-binding sites flanking the *white* gene stabilize *white* expression, making it independent of the $e(y)1^{u1}$ and z^{v77h} mutation combination. The su(Hw)-binding region in *gypsy* mobile element has the properties of an insulator: it interferes with expression of the gene in tissues where it is regulated by enhancers located distally from the su(Hw)-binding site with respect to the promoter (13, 23, 31, 32, 49, 53). Two su(Hw)-binding regions flanking a construction make the expression of a gene independent of the negative effect of a surrounding chromatin. It may be that su(Hw) insulators support an open chromatin structure in the promoter area of the mini-*white* gene that facilitates binding of the truncated TAF$_{II}$40 protein to the *white* promoter. However, further experiments are necessary to support this proposition.

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