TAF250 is required for multiple developmental events in *Drosophila*

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The TFIID transcription initiation complex is composed of TBP and multiple TAFs. Studies in unicellular systems indicate that TAF250 is required for progression through G_1/S of the cell cycle and repression of apoptosis. Here we extend these *in vivo* studies by determining the developmental requirements for TAF250 in a multicellular organism, *Drosophila*. *TAF250* mutants were isolated in a genetic screen that also yielded *TAF60* and *TAF110* mutants, indicating that TAFs function coordinately to regulate transcription. Null alleles of *TAF250* are recessive larval lethal. However, combinations of weak loss-of-function *TAF250* alleles survive to adulthood and reveal requirements for TAF250 during ovary, eye, ocelli, wing, bristle, and terminalia development as well as overall growth of the fly. These phenotypes suggest roles for TAF250 in regulating the cell cycle, cell differentiation, cell proliferation, and cell survival. Finally, molecular analysis of *TAF250* mutants reveals that the observed phenotypes are caused by mutations in a central region of TAF250 that is conserved among metazoan organisms. This region is contained within the TAF250 histone acetyltransferase domain, but the mutations do not alter the histone acetyltransferase activity of TAF250 *in vitro*, indicating that some other aspect of TAF250 function is affected. Because this region is not conserved in the yeast TAF250 homologue, TAF145, it may define an activity for TAF250 that is unique to higher eukaryotes.

TFIID is a transcription initiation factor that nucleates the assembly of RNA polymerase II and other initiation factors (TFIIA, TFIIB, TFIIE, TFIIF, and TFIIH) at the core promoter of protein-coding genes (1). The most abundant form of *Drosophila melanogaster* TFIID is composed of TBP and eight TAFs: TAF250, TAF150, TAF110, TAF80, TAF60, TAF40, TAF30α, and TAF30β (2). Similar stable multimeric TFIID complexes exist in all eukaryotic organisms investigated to date (3). In the yeast *Saccharomyces cerevisiae*, almost all TAFs are essential for viability (4). In *Drosophila*, *TAF40*, *TAF60*, and *TAF110*, the only TAFs that have been examined, are recessive embryonic or larval lethal and cell lethal, indicating the critical role that TAFs play *in vivo* (5, 6). However, it remains to be determined whether all TAFs or only subsets of TAFs are required for the transcription of TAF-dependent genes *in vivo*, whether TAFs are required for developmental events that are particular to multicellular organisms, and whether biochemical activities attributed to TAFs *in vitro* are required *in vivo*.

TAF250 (also designated TAF230 in *Drosophila*, CCG1 in humans, and TAF145/135 in yeast), is required for progression through the G_1/S boundary of the cell cycle. Mammalian or yeast cells carrying temperature-sensitive alleles of *TAF250* arrest in G_1 at the nonpermissive temperature (7, 8). Furthermore, following growth arrest, mammalian cells undergo apoptosis (9). In addition to serving as a scaffold on which other TAFs and TBP are assembled, TAF250 possesses enzymatic, promoter recognition, TBP regulatory, and coactivator activities. Transcriptional defects observed in these cells at the nonpermissive temperature presumably result from elimination of one or more TAF250 activities.

TAF250 contains two independent protein kinase domains and a histone acetyltransferase (HAT) domain. The kinase domains can autophosphorylate as well as transphosphorylate the largest subunit of TFIIF, RAP74, *in vitro* (10). The HAT domain can acetylate lysine residues in the N-terminal tails of histones H3 and H4 *in vitro* (11). Because acetylation of histones induces changes in chromatin structure, the HAT activity of TAF250 may provide a mechanism for TFIID to access transcriptionally repressed chromatin. The TAF250 bromodomains may contribute to this process because bromodomains have been shown to bind the N terminus of histone H4 and acetylated Lys residues (12, 13).

Analysis of gene expression patterns in yeast has shown that a subset of genes display a transcriptional requirement for TAF250 (14). Included are genes required for cell cycle progression such as G_1 phase cyclin genes as well as growth-related genes such as those encoding ribosomal proteins (15). Mutational analysis of several yeast genes has mapped the requirement for TAF250 to the region surrounding the TATA box.

The N-terminal region of TAF250 binds tightly to TBP and inhibits the ability of TBP to bind the TATA box and mediate basal transcription (16). TFIIA and transcriptional activators compete with TAF250 binding to TBP, suggesting that negative regulation of TBP by TAF250 may be an important regulatory step during transcription initiation (17, 18). Finally, a proposed coactivator role for TAF250 is based on the observation that TAF250 can interact with activator proteins such as adenovirus E1A (19). In summary, TAF250 has been shown *in vitro* to be a multifunctional component of TFIID, but the *in vivo* significance of many of these functions remains to be determined.

We have identified mutations in *Drosophila TAF250* in a genetic screen that is sensitive to transcription levels. The availability of loss-of-function mutations in *TAF250* has allowed us to determine how *Drosophila* development is affected by the absence of this broadly acting transcriptional regulator and define a protein domain that is critical for its function *in vivo*.

Materials and Methods

***Drosophila* Stocks and Genetic Screen.** The screen for dominant modifiers of *sev-Ras1*[V12] and the initial characterization of *SR3–5* alleles have been described (6). *EP(3)0421* and *Df(3)BD5* were obtained from the Bloomington Stock Center (Bloomington, IN) (20). *EP(3)0421* excision (*EX*) alleles were generated by mobilizing the *w*[+] P-element by crossing *EP(3)0421/TM3* flies with flies carrying a source of transposase. *cell*[1] was provided by J. Kennison (National Institutes of Health, Bethesda) (21).

Molecular Analysis of *EP(3)0421*. The site of P-element insertion in *EP(3)0421* and the nature of the lesions in *EP(3)0421*[EX1] and the *SR3–5* alleles were determined as described (22). Western blots of total ovary extracts were probed with a monoclonal TAF250 antibody (80H9) and a polyclonal TAF110 antibody (1309) provided by R. Tjian (University of California, Berkeley).

Examination of *TAF250* Mutant Phenotypes. *Scanning electron microscopy (SEM).* Adult flies (3- to 4-day-old males) were prepared for SEM by dehydration through an ethanol-in-water series (25, 50, 75,

100, 100%) and then a 1,1,2-trichlorotrifluoroethane (Freon)-in-ethanol series. Each step was for 12 h at room temperature. Flies were mounted on aluminum stubs and imaged on a Hitachi scanning electron microscope.

Eye sections. Sectioning of eyes was performed as described (23).

Ovary staining. Ovaries were dissected from 5-day-old flies and stained with 4′,6-diamidino-2-phenylindole as described (24).

Developmental time course. Crosses between flies of the appropriate genotype were maintained for 8 days before removal of the parents. Every 24 h over a 5-day period, progeny were removed and scored for *Sb* to differentiate heterozygous from transheterozygous flies. The percentage of transheterozygous flies is defined as the ratio of transheterozygous to total flies and was determined for each day and over the 5-day period (Fig. 3*N*). For example, over the 5-day period, 11% of the progeny from crosses between *TAF250*[XS-2232]/*TM3, Sb* and *TAF250*[EP421]/*TM3, Sb* flies were transheterozygotes, but on the first day only, 1% were transheterozygotes. Therefore, 9% (1/11%) of the expected value eclosed on the first day and it was not until the third day that 100% was achieved, indicating a 2-day delay in development. At least 1,400 flies were scored for each genotype.

HAT Assay. A recombinant protein encompassing amino acids 612–1,140 (middle region, M) of *Drosophila* TAF250 was produced in bacteria by using the pET expression system and purified according to the manufacturer's protocols (Novagen). Mutations identical to those identified in the *SR3–5* alleles were generated by mutagenesis of the pET-250-M expression plasmid using a site-directed mutagenesis kit (Stratagene).

HAT activity gel assays were performed as described (25). Equivalent amounts of protein were loaded in corresponding lanes of Fig. 5 *A* and *B*. Fig. 3*C* lane 7 contains less yeast HAT1 to equalize the signal with the TAF250 protein lanes. Yeast HAT1 protein was provided by P. Wade (National Institutes of Health, Bethesda).

Results

Signals Mediated by Ras1 Are Sensitive to Mutations in Components of the Basic Transcription Machinery.

Changes in the transcription profile of a cell occur in response to signals mediated by the Ras GTPase (25, 26). Much work has focused on the gene-specific enhancer-binding proteins that are direct targets of the Ras signal, but components of the transcriptional machinery that interact with enhancer-binding proteins presumably play equally important roles in regulating transcription. Consistent with this proposal, mutations in subunits of TFIIA and TFIID interfere with the ability of Ras1 to specify cell fate decisions during *Drosophila* eye development (6, 27).

Mutations in *TAF60* and *TAF110* were isolated in a genetic screen as suppressors of the rough eye phenotype caused by misexpression of a constitutively active form of Ras1 (Ras1[V12]) under transcriptional control of the *sev* enhancer and promoter elements (*sev-Ras1*[V12]). Details of the screen are described by Karim *et al.* (6). In brief, *sev-Ras1*[V12] transgenic flies have a rough eye phenotype that varies in severity in response to changes in Ras1 signaling strength. A screen was performed to identify dominant modifiers of this dose-sensitive phenotype. Genetic interaction studies were then used to categorize the isolated modifiers into two general groups: Class I, genes that function directly in the Ras1 signaling pathway, and Class II, genes that function to transcriptionally regulate the *sev-Ras1*[V12] transgene or downstream gene targets of the pathway. Class I groups have subsequently been shown to include protein kinases that function in a general Ras1 signaling cassette and the only Class II groups characterized thus far, *SR3–4B* and *SR3–3*, correspond to *TAF60* and *TAF110*. Thus, it appears likely that additional Class II groups (*SR3–2*, *SR3–4A*, *SR3–5*, *SR3–7*, and *SR3–8*) will encode transcriptional regulators. This study focuses on *SR3–5*.

TAF250	SR3-5[S-136]	SR3-5[S-625]	SR3-5[XS-2232]	cell[1]	EP(3)0421	EP(3)0421[EX1]	Df(3R)BD5
SR3-5[S-136]	FC						
SR3-5[S-625]	FC	FC					
SR3-5[XS-2232]	FC	FC	FC				
cell[1]	FC	FC	FC	FC			
EP(3)0421	C[a]	C[a]	C[a,b]	FC	C[a]		
EP(3)0421[EX1]	FC	FC	FC	FC	FC[c]	FC	
Df(3R)BD5	FC	FC	FC	FC	FC	FC	FC

Fig. 1. *TAF250* complementation analysis. Each of the *TAF250* alleles was crossed to the other alleles to determine whether they complement (C) or fail to complement (FC) the recessive lethality. *SR3–5* alleles were identified as suppressors of activated Ras1 (6), *cell*[1] was identified as a lethal mutation in the Antennapedia complex (21), *EP(3)0421* is a P-element insertion line (20), *EP(3)0421*[EX1] is a derivative of *EP(3)0421* in which the P-element has been excised, and *Df(3R)BD5* contains a chromosomal deletion that removes the *TAF250* gene. Transheterozygous flies containing the *EP(3)0421* allele display adult phenotypes: (*a*) female sterility and reduced bristle number on both male and female heads; (*b*) semilethal (11% of transheterozygous flies survive to adulthood; see Fig. 3*N*); and (*c*) female sterility.

The SR3–5 Locus Encodes Drosophila TAF250.

The ability of *SR3–5* alleles to suppress the *sev-Ras1*[V12] rough eye was mapped to meiotic position (3–47 ± 5). Chromosomal deletions *Df(3R)Scr* and *Df(3R)BD5* failed to complement the recessive lethality of all of the *SR3–5* alleles and suppressed the *sev-Ras1*[V12] rough eye, placing the gene within the polytene interval 84A1; 84B1–2 (Fig. 1; data not shown). This region includes the *TAF250* gene that maps to 84A2–3.

Searches of the Berkeley *Drosophila* Genome Project (BDGP) database identified a mutant line, *EP(3)0421*, in which a P-element is inserted within exon 1 of *TAF250*, 55 bp downstream of the translation start site (Fig. 2*A*) (20, 28). To determine if TAF250 expression is affected in *EP(3)0421* flies, ovary extracts were prepared from wild-type and *EP(3)0421* heterozygous and homozygous flies and probed with a TAF250 antibody (Fig. 2*B* and *Materials and Methods*). Relative to TAF110, TAF250 protein expression is drastically reduced in *EP(3)0421* homozygous flies.

EP(3)0421 is homozygous viable and complements the lethality of *SR3–5*[S-136] and *SR3–5*[S-625], but it only partially complements *SR3–5*[XS-2232] (Fig. 1). Approximately 11% of the progeny from crosses between *SR3–5*[XS-2232]/*TM3* and *EP(3)0421*/*TM3* flies are transheterozygotes (i.e., *SR3–5*[XS-2232]/*EP(3)0421*) which is less than the expected 33% if the transheterozygotes were completely viable (Figs. 1 and 3*N*).

To determine whether the phenotypes of *EP(3)0421*-containing flies are caused by insertion of the P-element in the *TAF250* gene and to create stronger *TAF250* alleles, the *EP(3)0421* P-element was excised by introducing a source of transposase. Viable lines in which the P-element was precisely excised no longer display bristle and sterility phenotypes observed in the parental line, indicating that the P-element was the cause of these phenotypes (data not shown). One of the lethal excision lines, *EP(3)0421*[EX1], was analyzed in detail. Sequencing of the *TAF250* gene from *EP(3)0421*[EX1] reveals that the parental P-element was internally deleted, leaving behind 52 bp of P-element sequence (Fig. 2*A*). The small insertion

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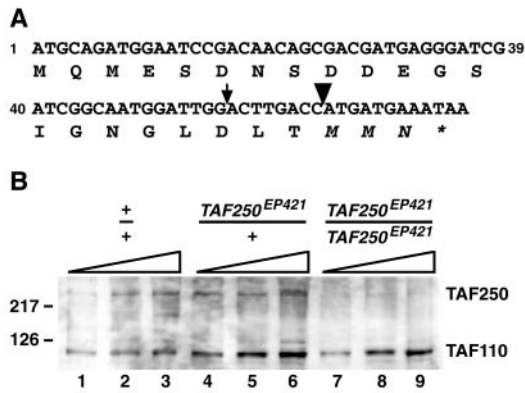
Fig. 2. Molecular characterization of *TAF250*[EP421] and *TAF250*[EP421-EX1]. (*A*) The arrow denotes the position of P-element insertion in *TAF250*[EP421] and the arrowhead denotes the position of sequence transition between *TAF250* and P-element sequence (in italics) in *TAF250*[EP421-EX1]. A stop codon (TAA), indicated by an asterisk, is contained within the P-element sequence that remains in *TAF250*[EP421]. (*B*) Extracts prepared from ovaries of flies of the indicated genotypes were probed with antibodies to TAF110 and TAF250. Triangles indicate increasing amount of extract. Molecular size markers in kDa are indicated on the left.

contains an in-frame stop codon within the first exon of *TAF250*, and therefore may be a null allele. In fact, like *Df(3R)BD5* which deletes the *TAF250* gene, *EP(3)0421*[EX1] fails to complement the lethality of all *SR3–5* alleles (Fig. 1).

In conclusion, the lethality observed with *SR3–5/EP(3)0421*[EX1] transheterozygotes and phenotypes observed with *SR3–5/ EP(3)0421* transheterozygotes (see below) indicate that the *SR3–5* locus corresponds to the *TAF250* gene. Therefore, we have renamed *EP(3)0421* and *SR3–5* mutants *TAF250* (i.e., *TAF250*[EP421], *TAF250*[EP421-EX1], *TAF250*[S-136], *TAF250*[S-625], and *TAF250*[XS-2232]).

***cell lethal* Is Allelic to *TAF250*.** To identify additional *TAF250* alleles, complementation analysis was performed with molecularly uncharacterized mutants that map near *TAF250*. One mutant, *cell lethal*[1] (*cell*[1]), failed to complement all of the *TAF250* alleles, indicating that, like *TAF250*[EP421-EX1] and *Df(3R)BD5*, *cell*[1] is a null allele of *TAF250* (Fig. 1) (21). *cell* was originally identified as a lethal complementation group in the Antennapedia complex. The lethal phase of *cell* and the *SR3–5* alleles occurs during the first to second larval instar, probably because of depletion of the maternal contribution of TAF250 (ref. 29 and data not shown). In view of the allelism with *TAF250*, we have renamed *cell* mutants *TAF250* (i.e., *cell*[1] is *TAF250*[1]).

***TAF250* Is Required for a Variety of Developmental Events.** Transheterozygous combinations of lethal *TAF250* alleles (*TAF250*[S-136], *TAF250*[S-625], *TAF250*[XS-2232], and *TAF250*[EP421-EX1]) and the viable P-element allele *TAF250*[EP421] exhibit developmental phenotypes. The severity and nature of these defects depends on the allelic combination (*TAF250*[XS-2232]/*TAF250*[EP421] > *TAF250*[S-136]/ *TAF250*[EP421] > *TAF250*[S-625]/*TAF250*[EP421] > *TAF250*[EP421]/ *TAF250*[EP421]) (Figs. 1 and 3 and data not shown). All allelic combinations display defects in oogenesis, ocelli development, and developmental timing; in addition, *TAF250*[XS-2232]/*TAF250*[EP421] flies display defects in eye, wing, bristle, and terminalia development, indicating roles for TAF250 in multiple developmental pathways.

TAF250 **is required for eye and ocelli development.** Mutations in *TAF250* disrupt development of photosensitive organelles, the eyes and ocelli. Adult compound eyes are composed of hundreds of ommatidia that are arranged in a perfect hexagonal array with single mechanosensory bristles that project from alternate vertices of ommatidia (Fig. 3*A*) (30). In *TAF250*[XS-2232]/*TAF250*[EP421] flies,

ommatidia are irregularly shaped and are not aligned in straight rows (Fig. 3*B*). In addition, the number of interommatidial bristles is reduced, multiple bristles sometimes originate from a single site, and bristles are randomly distributed.

The number of photoreceptor cells per ommatidium is altered in *TAF250* adult flies (Fig. 3*C*). Apical tangential sections through wild-type eyes reveal a trapezoid pattern of photoreceptors, with six large outer photoreceptors (R1–6) forming the perimeter of the trapezoid and one small inner photoreceptor (R7) in the center of the trapezoid (Fig. 3*C*) (29). In *TAF250*[XS-2232]/*TAF250*[EP421] flies, approximately 10% of the ommatidia are missing one outer photoreceptor and 14% have one extra outer photoreceptor (*n* = 270).

TAF250 mutations also disrupt development of ocelli. Three ocelli are normally arranged in a triangle on the top of the head (Fig. 3*D*). Severe *TAF250* mutants completely lack individual ocelli whereas less severe mutants have ocelli that are reduced in size (Fig. 3*E*).

TAF250 **is required for bristle development and patterning.** Mutations in *TAF250* alter bristle development on the thorax and head. The adult cuticle of *D. melanogaster* has numerous precisely positioned large bristles, macrochaete, and small bristles, microchaete (31). On the thorax, ten rows of microchaete bristles are positioned between pairs of macrochaete bristles (Fig. 3*F*). In *TAF250* mutants, several bristles in each row are shifted slightly, disrupting the orderly pattern of bristle rows (Fig. 3*G*). Microchaete size and shape do not appear to be affected and all aspects of macrochaete morphology appear normal on the thorax. In contrast, the number of bristles surrounding the ocelli is reduced, including complete elimination of ocellar bristles (Fig. 3*E*).

TAF250 **is required for terminalia development.** Mutations in *TAF250* result in incomplete rotation of the terminal A9 body segment in males. It has been hypothesized that during pupation, the developing terminalia undergo a 360° rotation in a clockwise direction to achieve the configuration of ejaculatory and tracheal ducts and the posterior peripheral nerves observed in adult male flies (Fig. 3*H*) (32). Incomplete rotation is observed in approximately 5% of *TAF250*[XS-2232]/*TAF250*[EP421] males (*n* = 90) (Fig. 3*I*). Different degrees of incomplete rotation occur. The most extreme phenotype is presented in Fig. 3*I* where the terminalia has rotated only 30° and the last tergite and analplate protrude from the body. It should be noted that this phenotype could be interpreted as a 30° overrotation of the terminalia.

TAF250 **is required for wing development.** Mutations in *TAF250* disrupt wing development. In *TAF250* flies, the fourth longitudinal vein (L4) bifurcates along the wing margin forming a delta (Fig. 3*K*). The shape and size of the wings appear normal as does the morphology of the other veins. However, the wings are often held out to the sides of the body, rather than crossed over the body (data not shown).

TAF250 **mutants are female sterile.** Mutations in *TAF250* cause female sterility but do not appear to affect the fertility of males. In the most severe case, *TAF250*[XS-2232]/*TAF250*[EP421], the ovaries are very small and the flies lay very few eggs that do not develop (data not shown). In a less severe case, *TAF250*[S-625]/*TAF250*[EP421], the ovaries appear normal in size and the flies lay more eggs but they do not develop. Examination of ovaries dissected from *TAF250*[S-625]/*TAF250*[EP421] flies reveals that approximately 5% of egg chambers contain 8 cells (7 nurse cells and 1 oocyte) in contrast to wild-type egg chambers that contain 16 cells (15 nurse cells and 1 oocyte) (*n* = 250) (Fig. 3 *L* and *M*). Eight-cell egg chambers may result from a reduction in the number of cell divisions, from four to three, that a single cystoblast normally undergoes to generate a 16-cell egg chamber (33).

TAF250 **is required for normal developmental timing.** Mutations in *TAF250* cause a delay in development (Fig. 3*N*). This was determined by examining how long transheterozygous *TAF250* mutants take to develop from an egg to adult relative to their heterozygous mutant siblings (see *Materials and Methods*). The

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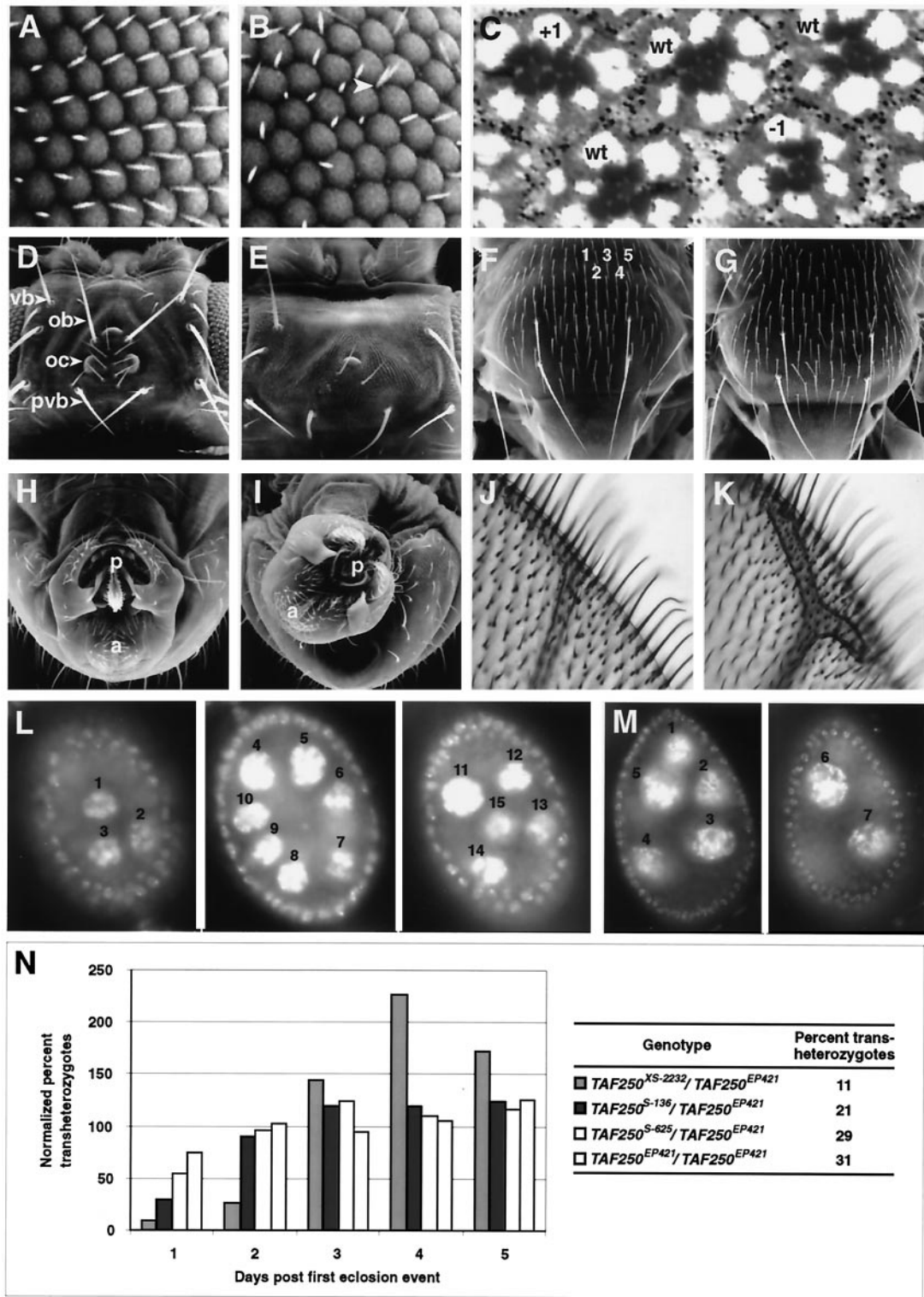
Fig. 3. Phenotypes of *TAF250* mutant flies. (*A*, *D*, *F*, *H*, *J*, and *L*) Wild-type flies. (*B*, *C*, *E*, *G*, *I*, and *K*) *TAF250*$^{XS\text{-}2232}$/*TAF250*^{EP421} flies; and (*M*) a *TAF250*$^{S\text{-}625}$/*TAF250*^{EP421} fly. (*A* and *B*) SEM of adult eyes. The arrow in *B* indicates two bristles that develop at a single position. (*C*) A light micrograph of an apical tangential section showing wild-type ommatidia (wt) containing seven photoreceptors and mutant ommatidia containing one extra (+1) or one fewer (−1) outer photoreceptor. (*D* and *E*) SEM of heads indicating ocelli (oc), vertical bristles (vb), ocellar bristles (ob), and postvertical bristles (pvb). (*F* and *G*) SEM of thoraxes indicating five of the bristle rows. (*H* and *I*) SEM of the terminal male body segment indicating the anus (a) and penis (p). (*J* and *K*) Light micrographs of wings focusing on the distal end of vein L4. (*L* and *M*) Staining of egg chambers with 4′,6-diamidino-2-phenylindole. Photographs were taken at several focal planes to image all of the nurse cell nuclei (which are numbered.) The oocyte nucleus is too small to detect. (*N*) The graph displays the percent of transheterozygous flies that eclose over time relative to the total percent of transheterozygous flies that eclose over the 5-day period (displayed in the table on the right).

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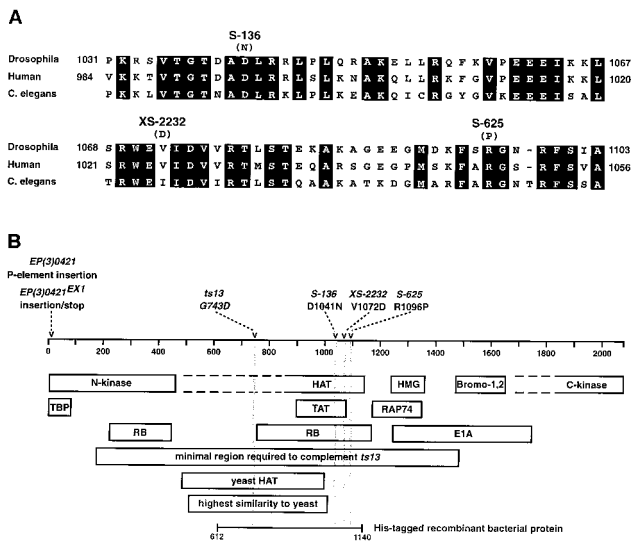
Fig. 4. Schematic representations of the identified mutations in TAF250. (*A*) Sequence comparison of a central region of *Drosophila*, human, and *Caenorhabditis elegans* with perfectly conserved amino acids highlighted (refs. 28 and 48; these sequence data were produced by the Sequencing Group at the Sanger Centre and can be obtained from ftp://ftp.sanger.ac.uk/pub). The *C. elegans* sequence is a conceptual translation of the genomic sequence so amino acid numbers are not available. The position and identities of amino acid changes in *TAF250* alleles are indicated above the sequence. (*B*) A comparison of the sites of mutations in TAF250, indicated above, and functional domains indicated below a line depicting the TAF250 protein. In addition to mutations identified in this study, the position of the temperature-sensitive *TAF250* mutation identified in the hamster cell line *ts13* is indicated (49). The N- and C-terminal kinase domains (10), the HAT domain (11), two bromodomains (28), a high mobility group (HMG) box (28), and binding domains for TBP (18), HIV TAT (34), RAP74 (10), retinoblastoma (35), and adenovirus EIA (19) are indicated. Additional regions are indicated that are minimally required to complement the phenotypes caused by growth of *ts13* cells at the nonpermissive temperature (50), display sequence similarity to the yeast TAF145 HAT domain (11), show the highest degree of sequence similarity to yeast TAF145 (51), and was used in the *in vitro* HAT assay.

strongest affect is observed with *TAF250*[XS-2232]/*TAF250*[EP421] flies that are delayed approximately two days in development, but all transheterozygous *TAF250* combinations are delayed to some extent. Delayed eclosion of mutant flies could reflect slowed developmental processes or an inability to gather enough nutrients.

The *SR3–5* Mutations Identify a Conserved Region of TAF250 That Is Critical for Its Function. *SR3–5* mutations should identify regions of the TAF250 protein that are important for its function *in vivo*. Therefore, the *TAF250*-coding region of the *SR3–5* alleles was sequenced (Fig. 4*A*). In each case, single amino acid changes were observed that lie within a 55-amino acid central region of TAF250 (Fig. 4*B*). The *TAF250*[S-136] gene product has an Asp-1041 → Asn change, *TAF250*[XS-2232] has a Val-1072 → Asp change, and *TAF250*[S-625] has an Arg-1096 → Pro change. These substitutions alter residues that are conserved within metazoan organisms but are not conserved in the homologous yeast protein TAF145 and thus potentially identify a TAF250 function that is specific to metazoan organisms.

The *SR3–5* Mutations in TAF250 Do Not Affect Its HAT Activity *in Vitro*. The point mutations in TAF250 fall within a region that has been shown to interact with transcriptional regulatory proteins, such as HIV TAT and retinoblastoma protein, and to possess HAT activity (Fig. 4*B*) (11, 34, 35). It should be noted that the TAF250 HAT domain does not contain sequence similarity to the acetyl-CoA binding site defined for acetyltransferases including GCN5 (36). To test whether HAT activity is altered by any of the TAF250
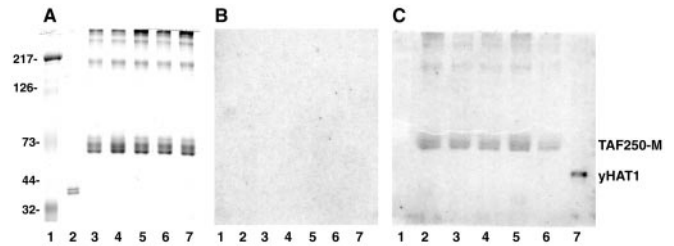
Fig. 5. Activity gel assays of a recombinant TAF250 protein fragment. (*A*) A Coomassie-stained gel. (*B*) An activity gel containing BSA. (*C*) An activity gel containing histones. (*A* and *B*) Corresponding lanes contain equivalent amounts of the same sample. Lanes: 1, protein markers (sizes in kDa indicated on the left); 2, recombinant yeast HAT1; 3, TAF250-M; 4, TAF250-M-C933Y; 5, TAF250-M-D1041N; 6, TAF250-M-V1072D; 7, TAF250-M-R1096P. (*C*) Lanes: 1, markers; 2–6, correspond to lanes 3–7 from *A* and *B*; 7, yeast HAT1.

mutations, a recombinant polypeptide encompassing amino acids 612–1,140 (TAF250-M) of *Drosophila* TAF250 was produced in bacteria, and site-directed mutagenesis was used to generate the identified single amino acid changes within the context of this polypeptide. Purified recombinant proteins were tested *in vitro* for HAT activity by an activity gel assay (25).

This assay revealed that the TAF250-M region of TAF250 has HAT activity, which narrows down the HAT domain to a smaller region than that defined by Mizzen *et al.* (11). Interestingly, the point mutations do not significantly affect this activity (Fig. 5*C*; compare lane 2 to 3–6). This result indicates that the mutations do not affect the catalytic activity of the TAF250 HAT, but it does not exclude the possibility that they regulate substrate specificity or the kinetics of the acetylation reaction.

Discussion

A Subset of TAFs Function Together to Regulate Transcription *in Vivo*. TAFs were originally defined biochemically as components of a complex, TFIID, that is required for activator-responsive transcription *in vitro* (2). Subsequent *in vitro* studies have shown that complexes containing only a subset of TAFs function similarly to holo-TFIID (37), and specific biochemical functions have been attributed to individual TAFs (3, 4). These findings raise the question whether all TAFs are required for the transcription of TAF-dependent genes *in vivo*. The isolation of mutations in *TAF60*, *TAF110*, and *TAF250* in the same genetic screen suggests that some genes require the function of multiple TAFs. This is supported by the finding that (*i*) *TAF60* and *TAF110* are required for the transcription of *twist* and *snail* genes in the *Drosophila* embryo (38); (*ii*) *TAF60* and *TAF250* mutants have similar adult phenotypes (N.A. and D.A.W., unpublished data); and (*iii*) the transcription of some genes in yeast depends on multiple TAFs (14).

Conversely, because all *Drosophila* TAFs were not isolated in the *sev-Ras1*[V12] screen, it suggests that not all TAFs are required for the transcription of TAF-dependent genes or that the dose sensitivities of TAFs are different. Unlike *TAF60*, *TAF110*, and *TAF250*, flies carrying deletions of *TAF30β*, *TAF80*, or *TAF150*, or specific mutations in *TAF40* do not suppress the rough-eye phenotype of *sev-Ras1*[V12] flies or the synthetic lethality of *sev-Ras1*[V12]/*Sev*[S11] flies (ref. 39, and data not shown). Taken together, these observations raise the possibility that there are multiple TFIID complexes that vary in the composition of TAFs and transcriptionally regulate specific genes.

The Phenotypes of *TAF250* Mutants Identify Potential Developmental Pathways and Genes Regulated by TAF250. The identification of allelic combinations of *TAF250* mutants that survive to adulthood has allowed us to examine the role of TAF250 during developmental events that occur after the early larval stages when homozygous *TAF250* null mutants die. The observed defects probably uncover

only a subset of TAF250 roles during development because, due to the method of isolation, the *TAF250* alleles may preferentially affect the transcription of genes involved in Ras1 signaling pathways.

TAF250 mutants were isolated as modifiers of a Ras1-induced phenotype and display phenotypes similar to those of flies mutant for components of Ras1 signaling pathways. In *Drosophila*, the epidermal growth factor receptor (EGFR) tyrosine kinase signals through Ras1 to control cell proliferation, survival, and differentiation. Similar to mutations in *TAF250*, mutations in *EGFR* or downstream components of EGFR signaling pathways cause female sterility, rough eyes with associated bristle defects, altered photoreceptor number, loss of ocelli and ocellar bristles, and extra wing vein material (40, 41). In addition, misrotated terminalia are observed in *head involution defective* (*hid*) mutant (42). *hid* encodes a pro-apoptotic factor that is transcriptionally regulated by the EGFR/Ras1 pathway (43, 44). The correlation between *TAF250* and EGFR pathway mutant phenotypes suggests that TAF250 transcriptionally regulates genes in the EGFR pathway (possibly *EGFR*, which is a target for TAF250 in mammalian cells, or downstream genes in the pathway like *hid*) (43, 45).

Alternatively, some of the *TAF250* phenotypes may result from altered transcription of ribosomal protein genes. *Minute* mutants encode ribosomal proteins and have common phenotypes, including delayed development, female sterility, and short and slender bristles (46). Furthermore, some *Minutes* have rough eyes, missing ocelli, or misrotated terminalia. *TAF250* mutants display all of these phenotypes, except for changes in bristle morphology, suggesting that, as in yeast, TAF250 regulates ribosomal protein gene transcription (15).

Finally, the oogenesis defect suggests a role for TAF250 in cell cycle progression, as has been reported for yeast and mammalian cells (7, 8). Mutations in *cyclin E,* which is necessary for the G_1/S transition, also cause the formation of eight-cell egg chambers, suggesting that TAF250 may regulate transcription of *cyclin E* (24).

In summary, defects in photoreceptor number suggest a role for TAF250 in cell fate determination, loss of ocelli and ocellar bristles and misrotation of terminalia suggest a role for TAF250 in promoting cell survival, the reduction in the number of cells per egg chamber suggests a role for TAF250 in cell cycle regulation, and the delay in development suggests a role for TAF250 in cell proliferation.

Mutations in *TAF250* Define a Region Critical for TAF250 Function *in Vivo*. Approximately 850,000 flies were scored in the *sev-Ras1*[V12] screen and three alleles of *TAF250* and six alleles of *TAF110* were isolated (6). All of the *TAF110* alleles contain point mutations, insertions, or deletions that lead to C-terminal truncations, whereas all of the *TAF250* alleles contain missense mutations that map to a 55-amino acid region of the 2,068 amino acid protein (E.M.S. and D.A.W., unpublished data). These results indicate that, unlike TAF110, which requires deletion of the C-terminal half of the protein for functional inactivation, TAF250 is inactivated by single amino acid changes in a small region of the protein. The challenge now is to determine the *in vivo* activity of this region. Whereas this region does not appear to affect HAT activity, it may regulate substrate specificity *in vivo*. In addition to histones, TAF250 can acetylate TFIIEβ and TFIIF *in vitro*; the physiological substrates of TAF250 have not been determined (47). Alternatively, this region may be required for stability of the TFIID complex or for interactions with other components of the transcription machinery, possibly factors such as HIV TAT or retinoblastoma which bind TAF250 *in vitro* (34, 35).

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