Drosophila TFIIA-S is up-regulated and required during Ras-mediated photoreceptor determination

Martin P. Zeidler,¹ Kyoko Yokomori,² Robert Tjian,² and Marek Mlodzik^{1,3}

¹Differentiation Programme, European Molecular Biology Laboratory, 69117 Heidelberg, Germany; ²Howard Hughes Medical Institute, Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-3202 USA

Photoreceptor induction in the developing *Drosophila* eye is triggered by the activation of the Ras pathway. Subsequently, the Ras-mediated activation of site-specific transcription factors leads to the expression of putative "effector" genes. The coactivator function of the basal transcription factor TFIIA has been shown previously to enhance the *trans*-activation potential of site-specific transcription factors in vitro. Here, we show that the expression of the small subunit of TFIIA (dTFIIA-S) is specifically up-regulated in a transient manner during Ras-mediated photoreceptor induction. Furthermore, although null mutations in *dTFIIA-S* are cell lethal, a hypomorphic *dTFIIA-S* allele demonstrates an increased requirement for this factor during photoreceptor development. In addition, the cone cell to R7 photoreceptor transformation caused by ectopic activation of the Ras pathway during eye development is suppressed by the removal of one functional copy of the dTFIIA-S locus revealing the sensitivity of this process to reductions in dTFIIA-S activity. These results are the first in vivo evidence for the coactivator function in transcriptional enhancement proposed for TFIIA.

[Key Words: Basal transcription machinery; eye development; *Drosophila*; Ras pathway; coactivators]

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The development of photoreceptors in the *Drosophila* eye proceeds by a series of successive recruitment steps mediated by intercellular signals (for reviews, see Dickson and Hafen 1994; Zipursky and Rubin 1994). These inductive cues cause undetermined epithelial cells that make up the eye imaginal disc to develop into specialized, light sensitive neurons. The stepwise recruitment of these photoreceptors requires the activation of the Ras pathway by membrane-bound receptor tyrosine kinases (RTKs). Two RTKs have been identified as fulfilling this role in the developing eye. The *Drosophila* epidermal growth factor (EGF) receptor homolog *Egfr* (Baker and Rubin 1992; Xu and Rubin 1993; Freeman 1994) and its putative ligand, the TGF α homolog *spitz*, are required for the induction of the future outer photoreceptors R1–R6 (Rutledge et al. 1992; Freeman 1994; Tio et al. 1994). A second RTK, Sevenless (Sev), is expressed in the future R7 cell and the outer photoreceptors R1/R6 and R3/R4 but is only activated by its ligand [encoded by *bride-of-sevenless* (*boss*); Krämer et al. 1991] in the R7 precursor (Dickson and Hafen 1994; Zipursky and Rubin 1994). In addition, it is also expressed in the nonneuronal cone cells and the so-called mystery cells (Tomlinson et al. 1987). Activation of Sev or any of the downstream signal transduction elements is sufficient to induce R7 fate in any of these nonneuronal cells. Thus, the cells consti-

tuting this group have been termed the R7 equivalence group (Dickson et al. 1992a).

Ligand-mediated autophosphorylation of either RTK results in the activation of the Ras GTPase and its subsequent kinase cascade including Raf kinase and MEK (or MAPKK encoded by *Dsor*) culminating in the phosphorylation of the *Drosophila* MAPK homolog Rolled (Biggs et al. 1994; Dickson and Hafen 1994; Zipursky and Rubin 1994). Rolled then translocates to the nucleus where it phosphorylates various site-specific transcription factors including Pointed, Yan, and Jun (Bohmann et al. 1994; Brunner et al. 1994a; O'Neill et al. 1994; Rebay and Rubin 1995). These factors then cause the expression of the effector genes thought to be required for the photoreceptor developmental program and cellular differentiation.

Transcription of the effector genes depends on the activity of both site-specific transcriptional activators (i.e., Jun and Pointed) and the basal transcription machinery. The interaction of transcriptional activators with components of the basal machinery is thought to cause the assembly of the mature protein complex on the promoter of its target genes. This complex consists of RNA polymerase II and several general transcription factors including TFIIA and TBP and TBP-associated factors (TAFs) (for review, see Maldonado and Reinberg 1995). Although relatively few of the components making up the basal machinery are required for low-level basal transcription in vitro, efficient activation of transcription requires the

³Corresponding author.

presence of additional factors (Chen et al. 1994). Thus, higher levels of transcription are dependent on the interaction of transcriptional activators with specific components of the basal machinery, subsequently termed coactivators (Pugh and Tjian 1990; Goodrich and Tjian 1994). Although a number of TAFs have been identified as fulfilling a coactivator function (including dTAF_{II}110 and dTAF_{II}40; Pugh and Tjian 1990; Gill et al. 1994; Goodrich and Tjian 1994), it has been reported that the basal factor TFIIA can also enhance levels of transcription in an activator-dependent manner (Goodrich et al. 1993; Hori and Carey 1994; Ozer et al. 1994; Sun et al. 1994; Yokomori et al. 1994). The identification and cloning of the components of the TFIIA complex have shown that the recombinant proteins not only increase the affinity of TBP for the TATA box and its stability once bound, but also significantly enhance the *trans*-activation potential of NTF and VP16 in vitro (Sun et al. 1994; Yokomori et al. 1994).

Here, we demonstrate that the small subunit of the TFIIA complex (*dTFIIA-S*) is expressed in a dynamic pattern during the development of the *Drosophila* eye. It is up-regulated in the photoreceptor precursors preceding and independent of Ras pathway activation during the time when their determination is induced by Ras activation. Although null alleles of *dTFIIA-S* are cell lethal, partial loss-of-function alleles show photoreceptor-specific disruptions in eye development. In addition, we show that the rough-eye phenotype and supernumerary R7 development caused by ectopic activation of the Ras pathway are suppressed by a reduction in the gene dose of *dTFIIA-S*. Taken together, we provide genetic evidence that indicates that full levels of effector gene expression stimulated by the Ras pathway in photoreceptor precursors in vivo depend on TFIIA.

Results

Cloning of *dTFIIA-S*

The *Drosophila* TFIIA-S (*dTFIIA-S*) transcription unit was identified by virtue of its proximity to an eye-specific enhancer detector P element and its sequence homology to yeast TFIIA-b. Genomic DNA was recovered by plasmid rescue of the F125 P element located at 95C7-8 (Mlodzik and Hiromi 1992) with its insertion site 1.6 kb 3' of the subsequently identified TFIIA-S coding region (Fig. 1). Overlapping genomic and cDNA clones were isolated from imaginal disc and embryonic libraries. The sequence of both cDNA and genomic clones was determined (EMBL library accession no. X83271) and found to encode a protein with homology to yeast TFIIA-b (Ranish et al. 1992). Subsequent biochemical analysis confirmed that the gene product is indeed the small subunit of *Drosophila* TFIIA (Yokomori et al. 1994).

dTFIIA-S is dynamically expressed during development

Although the 700-nucleotide-long *dTFIIA-S* transcript is

Figure 1. Genomic organization of the *dTFIIA-S* locus. The genomic region containing the *dTFIIA-S* transcription unit is shown. The coding region is indicated by the shaded region. The gene contains a single 57-bp intron. Genomic deletions associated with imprecise excisions of the adjacent F125 P element, *dTFIIA-S*^{E73}, *dTFIIA-S*^{E69}, *dTFIIA-S*^{E31}, *dTFIIA-S*^{E89}, *dTFIIA-S*^{E32}, and *dTFIIA-S*^{E62} are shown below the genomic map; black bars represent deleted DNA, and grey bars represent the limits of uncertainty inherent to the mapping process. The 4-kb genomic region lying between the two *EcoRI* (R) restriction sites was used for rescue experiments. The size of the F125 P element is not to scale. Restriction enzyme sites are abbreviated as follows: (Xb) *Xba*I, (B) *Bam*HI, (R) *Eco*RI, (P) *Pst*I, (N) *Nru*I, and (H) *Hpa*I.

present at high levels throughout *Drosophila* development (as detected by Northern and in situ hybridization analysis; not shown), a detailed analysis of the *dTFIIA-S* expression as shown using a specific polyclonal rabbit antiserum (Yokomori et al. 1994) reveals a dynamic expression pattern. Maternally supplied *dTFIIA-S* protein is initially distributed uniformly in fertilized eggs (not shown) and becomes localized to all nuclei during the syncytial cleavage stages. All nuclei show fairly uniform protein expression just prior to the cellular blastoderm stage (Fig. 2A). Although *dTFIIA-S* distribution during later embryonic development remains largely uniform, some cell groups, including delaminating neuroblasts and cells of the mesodermal layers in stage 7 embryos, express higher levels with the onset of differentiation (Fig. 2B).

During imaginal disc development in third-instar larvae, *dTFIIA-S* is also detected at low levels in all nuclei. However, increased expression is apparent in specific regions within imaginal discs. In particular, third-instar eye imaginal discs show a striking pattern of high level *dTFIIA-S* expression posterior to the morphogenetic furrow (MF; Fig. 2E). The temporal sequence of cellular recruitment during ommatidial assembly is visible as distinct columns of developing ommatidial clusters posterior to the MF in a single eye imaginal disc (Tomlinson and Ready 1987), therefore allowing the dynamic temporal expression pattern of *dTFIIA-S* to be followed. Clusters of cells expressing *dTFIIA-S* are first identified in the MF in column 1 corresponding to the R8 and R2/R5 precursors (Fig. 2F). Slightly more posterior in column 3, increased expression is detected in precursors corresponding to R8, R2/R5, and R3/R4 (Fig. 2G). Subsequently, in columns 4/5, expression fades in R8 and R2/R5 and becomes stronger in R3/R4 (Fig. 2H). By col-

Figure 2. Embryos and eye imaginal discs stained with an anti-dTFIIA-S. A polyclonal rabbit antiserum raised against bacterially expressed dTFIIA-S protein is used in all panels. Anterior is to the left, and dorsal is up. (A–D) Embryonic stages; (E–L) eye imaginal discs. (A) Syncytial blastoderm embryo. dTFIIA-S is localized to the nuclei during syncytial nuclear divisions and is largely provided maternally at this stage. (B) Embryo at germ-band extension (approximately stage 8). Delaminating neuroblasts (white arrowhead) contain higher levels of dTFIIA-S than the overlying ectodermal cell layer. The mesoderm also exhibits higher levels of dTFIIA-S at this stage (white arrow). (C,D) Stage 15 embryos. In wild type (C), dTFIIA-S is detected in all nuclei. The developing central nervous system appears to be stained more strongly. However, it is possible that the increased dTFIIA-S staining evident in the CNS at this stage may be a consequence of the higher cell density in this tissue. (D) Homozygous mutant *dTFIIA-S^{E32}* embryo. No dTFIIA-S protein is detectable at this stage, and development does not proceed beyond this point. (E) dTFIIA-S expression in the third-instar eye imaginal disc. Although low-level expression is maintained in all cells, patterned increased expression is detected posterior to the MF (indicated by black arrowhead). (F–K) High-magnification photomicrographs of progressive changes in dTFIIA-S expression pattern posterior to the MF. (F) Clusters of photoreceptor cells expressing dTFIIA-S are first identified in the MF in column 1 corresponding to R8 and R2/R5. (G) In column 3, the R3/R4 cells join the R8, R2/R5 cluster. (H) In column 4/5, expression fades in R8 and R2/R5 and gets stronger in R3/R4. (I) In column 6, expression is strong in R3/R4 and R1/R6. (J) In column 7, expression has faded in R3/R4 and R1/R6, and R7 expression begins. (K) Expression in cone cells (c) is first initiated in column 7/8 and is maintained to the posterior of the disc. It is detected in all four cells (column 11 is shown). (L) Expression in the R7 precursor in the *sev^{d2}* mutant background. Expression in the cell occupying the R7 precursor position (7') is still up-regulated, despite associated loss of Sevenless/Ras signaling in this cell.

umn 6, expression continues in R3/R4 and is up-regulated in R1/R6 (Fig. 2I). One column further posterior expression is initiated in R7 (Fig. 2J). Finally, the four nonneuronal cone cells (column 11; Fig. 2K) begin to express high levels of dTFIIA-S that is then maintained to the posterior of the eye disc (Fig. 2E), whereas that in the photoreceptor precursors returns to basal levels. Note that dTFIIA-S expression is always nuclear and transient in all photoreceptor cells of the developing ommatidial cluster.

In addition to this dynamic pattern in photoreceptor precursors, elevated expression of dTFIIA-S is also detected in two bands ahead of and immediately behind the MF. Nuclei in these stripes are rounded, apically positioned, and unusually large and may represent cells dividing during the first and second mitotic waves (Tomlinson and Ready 1987).

dTFIIA-S levels peak as the Ras pathway determines cell fates

To ascertain the developmental stage at which the peak

of dTFIIA-S expression in each photoreceptor precursor occurs (shown in Fig. 2F–K), double-labeling experiments using antibodies specific for dTFIIA-S and the neuronal marker Elav were carried out. The nuclear antigen Elav (Robinow and White 1991) is one of the first neuronal markers to be expressed in developing and differentiating photoreceptor cells. The beginning of high level dTFIIA-S expression precedes that of Elav by 2–3 hr (one to two columns; Fig. 3). For instance, R8 and R2/R5 express high levels of dTFIIA-S (shown in red in Fig. 3) in column 1, behind the MF (Fig. 3B), ~2 hr before coexpression with Elav (shown in green) is detected (Fig. 3C). Similarly, dTFIIA-S is also apparent in other precursors prior to Elav expression (Fig. 3D–F). Shortly after a particular precursor begins to differentiate, dTFIIA-S expression returns to the basal level (Figs. 2E and 3A, data not shown). Thus, in each photoreceptor precursor cell, high dTFIIA-S levels are only detected for 6–10 hr, representing three to five columns. Significantly, the increase in dTFIIA-S expression in R7 (Fig. 3F) coincides precisely with the period during which Sev/Ras signaling is required to induce this cell fate (Basler and Hafen 1989;

Figure 3. Double immunofluorescence of a wild-type eye imaginal disc stained for dTFIIA-S (red) and the neuronal marker Elav (green). Cells in which both proteins are expressed are thus stained yellow. Note that dTFIIA-S is up-regulated at least one column anterior to Elav expression in each photoreceptor cell type. The precursor cells first express only dTFIIA-S (red), followed by overlapping expression of both dTFIIA-S and Elav (yellow colored cells). Subsequently, dTFIIA-S expression is down-regulated in the respective precursors. However, note that all cells constitutively express low levels of dTFIIA-S. The most anterior portion of the eye imaginal disc shown is in A, top left. (B–F) Detailed analysis of double-stained photoreceptor clusters. The cells are labeled with the respective photoreceptor numbers to identify cell type. (B) dTFIIA-S expression in column 1 in R8 and R2/R5. (C) R8, R2/R5 cells in column 2–3 (~2 hr later). R2/R5, and shortly afterwards R8 as well, express both dTFIIA-S and Elav. (D) Columns 4/5: dTFIIA-S expression has been up-regulated in R3/R4 (and just beginning in the R1/R6 pair), whereas it has been down-regulated in R8 and R2/R5. Elav expression is maintained in R8 and R2/R5 (green). (E) Column 6: R3/R4 express both dTFIIA-S and Elav, whereas R1/R6 still express predominantly dTFIIA-S (red). R8 and R2/R5 are no longer in this focal plain. (F) Column 7/8: The future R7 expresses just dTFIIA-S (red), whereas R3/R4 and R1/R6 coexpress dTFIIA-S and Elav (yellow).

Mullins and Rubin 1991). Moreover, the temporal regulation of high levels of dTFIIA-S in the other R-cell precursors also correlates with the time window during which they are thought to be determined and require the Ras pathway (for review, see Dickson and Hafen 1994; Zipursky and Rubin 1994). Thus, the highest levels of dTFIIA-S are observed in photoreceptor precursors during their Ras-mediated induction and determination. To examine the possible relationship between neuronal induction and up-regulation of dTFIIA-S, *sev*[−] mutant eye imaginal discs were examined. Although the R7 photoreceptor precursor fails to assume a neuronal identity in this genetic background, dTFIIA-S expression is still up-regulated in the mutant R7 precursor cell as in the wild-type situation (Fig. 2, cf. J and L). Thus, the up-regulation of dTFIIA-S expression occurs via a mechanism independent of the Ras pathway.

In contrast to the small subunit, the levels of expression of the large subunit of TFIIA (dTFIIA-L) do not ap-

pear to be modulated during photoreceptor development (not shown).

Mutations in *dTFIIA-S*

To investigate potential developmental requirements of *dTFIIA-S*, both null alleles and a hypomorphic allele (*dTFIIA-S*^{E73}) were generated by remobilization of the adjacent P element (Mlodzik and Hiromi 1992) thereby introducing small deletions in the locus through imprecise excision events (Fig. 1; Daniels et al. 1985). In the null alleles *dTFIIA-S*^{E69} and *dTFIIA-S*^{E31}, all genomic DNA between the P element and ~1 kb upstream of *dTFIIA-S* is removed. The hypomorphic allele *dTFIIA-S*^{E73} lacks genomic DNA lying between the original insertion site and the 3' end of the transcript (Fig. 1). Three smaller deletion alleles were also recovered that remove just the *dTFIIA-S* coding region (*dTFIIA-S*^{E89}, *dTFIIA-S*^{E32}, and *dTFIIA-S*^{E62}). These may have resulted from a local reinsertion of F125 into the 5' region of *dTFIIA-S* followed by a subsequent imprecise excision.

Embryos homozygous for the null allele *dTFIIA-S*^{E32} die at stage 15–16 of embryogenesis and contain no detectable dTFIIA-S protein at this stage (Fig. 2, cf. C and D). Mutant embryos appear to develop normally until this stage, and no significant differences in dTFIIA-S levels in mutant and wild-type embryos are detected until shortly before death. It is therefore likely that maternally supplied dTFIIA-S is sufficient to allow embryonic development up to this stage. No clones of mutant cells homozygous for null alleles of *dTFIIA-S* are ever recovered in third-instar eye discs, adult eyes, or cuticle, although twin spot regions are produced at high frequencies and thus recombination events have been induced (not shown). This indicates that dTFIIA-S is required for cellular survival. Animals homozygous for the hypomorphic allele *dTFIIA-S*^{E73} survive to pharate adult stages, and clones homozygous for this allele are recovered at normal frequency in adult tissues (see below).

To prove that the lesions in the *dTFIIA-S* locus are the sole source of lethality on the respective chromosomes, the phenotypes were rescued by *dTFIIA-S* transgenes. The 4-kb *EcoRI* genomic fragment including the *dTFIIA-S* coding region and P-element insertion site (Fig. 1) or a *dTFIIA-S* cDNA expressed from the *hsp70* promoter were sufficient to rescue the *dTFIIA-S*^{E32} and *dTFIIA-S*^{E73} alleles to adulthood. These chromosomes therefore contain only disruptions at this locus, and thus, the observed phenotypes are attributable solely to a lack of *dTFIIA-S* function (Fig. 5; data not shown).

dTFIIA-S is required for eye development

The dynamic expression pattern of dTFIIA-S in the developing eye imaginal disc prompted us to test for the potential role of *dTFIIA-S* during eye development. Because null alleles are cell lethal (see above), we analyzed the eye phenotype of the hypomorphic *dTFIIA-S*^{E73} allele. Larval eye imaginal discs homozygous for *dTFIIA-S*^{E73} are similar in size to wild type, and neuronal anti-

Figure 4. The phenotype of the *dTFIIA-S*^{E73} allele in homozygous mutant eye imaginal discs. (A,B) Eye imaginal discs stained with an anti-BarH1 antiserum serving as an R1/R6 cell type marker. (A) *dTFIIA-S*^{E73} mutant disc; (B) wild-type control disc. Note in A that the pairs of R1/R6 cells are irregularly arranged and examples of ommatidia lacking one (black arrows) or both (white arrows) are present; compare with the regular arrangement of R1/R6 pairs within the developing wild-type preclusters (B). (C–D) Eye imaginal discs stained with an anti-β-galactosidase antibody heterozygous for the enhancer–detector insertion H214 that marks developing R7 cells. (C) *dTFIIA-S*^{E73} mutant disc; (D) wild-type control disc. Note that in the mutant discs (C), ommatidial preclusters with multiple staining cells (black arrows) and also some lacking an R7 (white arrow) are found.

gens are expressed. However, disruptions in photoreceptor number, identity, and position within developing clusters are apparent. Third-instar eye imaginal discs homozygous for *dTFIIA-S*^{E73} were stained for expression of the BarH1 protein, an R1/R6 marker (Higashijima et al. 1992). In contrast to the regular repeated pattern of R1/R6 cells in wild-type discs (Fig. 4B), mutant eye discs show a severely disrupted pattern in which clusters are often lacking either one (black arrows) or both (white arrows) R1/R6 type cells (Fig. 4A). Similarly, the normal pattern of R7 cells, as visualized by the enhancer–detector line H214 (Mlodzik et al. 1992; Fig. 4D), is disrupted in *dTFIIA-S*^{E73} homozygous discs and ommatidia with multiple H214-expressing cells are present (black arrows in Fig. 4C; see also Fig. 5A).

In adult eyes, clones homozygous for the *dTFIIA-S*^{E73} allele show severely disrupted ommatidia characterized by frequent loss of photoreceptors. In addition, ommatidia are found that contain additional R7 cells coinciding with the loss of outer R1–R6 cells, further suggesting that a transformation of R1–R6 to R7 type photoreceptors has occurred (Fig. 5A, arrowheads; see also Fig. 4). Flies homozygous for the *dTFIIA-S*^{E73} allele rescued to adulthood by a single copy of the *dTFIIA-S* cDNA under the control of the *hsp70* promoter (see above) have wild-type eye morphology (Fig. 5B). Thus, the disruption observed in *dTFIIA-S*^{E73} eye clones is a direct conse-

quence of a reduction in *dTFIIA-S* levels. The *dTFIIA-S* mutant phenotypes illustrate the absolute requirement of *dTFIIA-S* for cellular survival and that photoreceptor determination and development are especially sensitive to a reduction in *dTFIIA-S* activity.

Cone cell transformation mediated by activated Ras pathway components is sensitive to dTFIIA-S gene dosage

Constitutively active forms of Sev, Ras1, or Raf expressed in cone cell precursors or the *Sevenmaker* (*Sem*) gain-of-function allele of *rolled*/MAPK transform the normally nonneuronal cone cell precursors to functional R7 neurons (Basler et al. 1991; Dickson et al. 1992b; Fortini et al. 1992; Brunner et al. 1994b). The resulting cell fate transformation causes an externally visible dominant rough-eye phenotype (Fig. 6A,E), the severity of which can be modulated by altering the levels of interacting factors, an approach successfully used in genetic screens for downstream effector genes (Biggs et al. 1994; Chang et al. 1995; Dickson et al. 1995).

To further examine the requirement for *dTFIIA-S* in photoreceptor induction, *dTFIIA-S* alleles were tested for dosage-sensitive interactions with rough-eye phenotypes caused by ectopic activation of Ras pathway components. Strikingly, removal of one functional copy of *dTFIIA-S* partially suppresses the formation of the ectopic R7 cells. This is illustrated both by the greater regularity of external eye morphology and the reduced number of R7 photoreceptors present in sections of such eyes (Fig. 6; Table 1). The degree of suppression produced by *dTFIIA-S*^{E32}, *dTFIIA-S*^{E73}, and the independent *dTFIIA-S*^{l(3)6624} (Karpen and Spradling 1992) alleles are similar in all genetic backgrounds tested (Table 1). Furthermore, this effect is specific for activated components of the Ras pathway and cone cell to R7 transformation.

Figure 5. Adult eye phenotype of the *dTFIIA-S*^{E73} allele. (A) Homozygous mutant clone. Mutant cells are marked by the absence of pigment granules. Photoreceptor arrangement is disrupted in mutant ommatidia. Note ommatidia with missing photoreceptors or extra R7 cells (black arrowheads), possibly resulting from an R1–R6 to R7 transformation as some outer photoreceptors are missing from such clusters (see also Fig. 4). (B) Section through the adult eye of an individual homozygous mutant for the hypomorphic allele *dTFIIA-S*^{E73} rescued by expression of the *dTFIIA-S* cDNA from the *hsp70* promoter. The internal morphology of the eye is essentially wild type.

Figure 6. Genetic interaction of *dTFIIA-S* alleles and activated Ras pathway components. Suppression of *sev*^{S11} (Basler et al. 1991; A–D), *sev-ras*^{V12} (Fortini et al. 1992; E,F), and *rolled*^{Sem} (Brunner et al. 1994b; I,J) phenotypes by gene dosage reduction in *dTFIIA-S* is shown. (Top) Control eyes; (bottom) *dTFIIA-S*^{E73} + siblings. Both the null allele *dTFIIA-S*^{E32} and the hypomorphic allele *dTFIIA-S*^{E73} show a similar suppression, whereas the original F125 chromosome is neutral. (A,B,E,F) Scanning electron micrographs (SEMs); (C,D,G–J) apical retinal sections at the R7 level. R8 is below this plane of section. Note the considerable reduction in external roughening and the more regular array of ommatidial clusters, with fewer ectopic R7 cells, in *dTFIIA-S* heterozygotes (bottom). Ommatidia with a wild-type photoreceptor complement are often recovered in such genotypes (D,H,J), whereas they are only very rarely found in control siblings (C,G,I). All eyes shown are from males. (A,C) *sev*^{S11}; +/+ (containing 4.06 ± 0.20 R7 cells per ommatidium, $n = 254$). (B,D) *sev*^{S11}; *dTFIIA-S*^{+/+} (containing 2.62 ± 0.14 R7 per ommatidium, $n = 836$). (E,G) *sev-ras*^{V12}, *CyO* +/+; +/+ (containing 2.46 ± 0.38 R7 per ommatidium, $n = 626$). (F,H) *sev-ras*^{V12}, *CyO* +/+; *dTFIIA-S*^{+/+} (containing 1.94 ± 0.05 R7 per ommatidium, $n = 1057$). (I) *rolled*^{Sem}/+/+; +/+ (containing 3.13 ± 0.55 R7 per ommatidium, $n = 618$). (J) *rolled*^{Sem}/+/+; *dTFIIA-S*^{+/+} (containing 2.52 ± 0.25 R7 per ommatidium, $n = 833$).

Table 1. Genetic interactions of *dTFIIA-S* alleles and Ras-mediated rough-eye phenotypes

	<i>dTFIIA-S</i> ^{E32}	<i>dTFIIA-S</i> ^{E73}	<i>dTFIIA-S</i> ¹⁽³⁾⁶⁶²⁴	1(3)Rpl1140 ^{Z3}	Df(21)PuD17
<i>se-poxn</i>	–	–	–	N.D.	N.D.
<i>sev-rough</i>	–	–	–	N.D.	N.D.
<i>sev</i> ^{S11}	++	+++	+	–	–
<i>se-raf</i> ^{torY9}	+	+/-	N.D.	N.D.	N.D.
<i>sev-ras</i> ^{V12}	++(+)	+++	++(+)	–	–
<i>rolled</i> ^{Sem}	++	+	N.D.	N.D.	N.D.
2x <i>sev-svp2</i>	+++	++	+++	N.D.	–

The rough-eye phenotype of the genotypes indicated was scored, and a subjective rating of observed suppression with relation to siblings and parental strains was generated. No suppression (–); the number of + symbols indicates progressively better suppression; (N.D.) not done. The slight variation in suppression observed between the *dTFIIA-S*^{E32} and *dTFIIA-S*^{E73} alleles tested is probably attributable to inherent variation of the rough-eye phenotypes tested. The independent *dTFIIA-S*¹⁽³⁾⁶⁶²⁴ allele (Karpén and Spradling 1992) was also tested for genetic interactions, as were point mutations in the RNA polymerase II 140-kD subunit [1(3)Rpl1140^{Z3}; Parkhurst and Ish-Horowitz 1991] and the deficiency Df(2L)PuD17 that removes the region encoding the TBP subunit of TFIID (Hoey et al. 1990). Note that only phenotypes generated by activation of components of the Sev/Ras pathway are sensitive to *dTFIIA-S* dosage; the *sev-poxn* and *sev-rough* genotypes are not affected. The genotypes tested were *sev-poxn*, Poxneuro homeo domain protein expressed by the *sev* enhancer and *hsp70* promoter (K. Basler, E. Hafen, and M. Noll, unpub); *sev-rough*, Rough homeo domain protein expressed by the *sev* enhancer and promoter (Basler et al. 1990; Kimmel et al. 1990); *sevenless*^{S11} (*sev*^{S11}), a constitutively activated Sevenless receptor (Basler et al. 1991); *se-raf*^{torY9}, constitutively active Raf expressed by the *sev* enhancer (Dickson et al. 1992b); *sev-ras*^{V12}, a constitutively activated allele of Ras1 expressed from the *sev* enhancer/promoter (inserted into the *CyO* balancer chromosome) (Fortini et al. 1992); *rolled*^{Sevenmaker} (*rolled*^{Sem}), a gain-of-function allele of the *Drosophila* MAP kinase *rolled* gene (Brunner et al. 1994b); and 2x *sev-svp2*:*seven-up* type II (two copies) expressed by the *sev* enhancer/promoter (Hiromi et al. 1993).

No genetic interaction is observed with other dosage-sensitive rough-eye genotypes including others that depend on *sev* enhancer-driven expression in the cone cells [e.g., *sev-rough* and *sevE-poxn* (Basler et al. 1990; Kimmel et al. 1990); see Table 1]. In addition, constructs carrying either the *sev* enhancer/*hsp70* promoter (e.g., *sev*^{S11} and *sE-ra*^{forY9}) or using both the *sev* enhancer and promoter (*sev-ras*^{V12} and *sev-svp2*) are affected by mutations in *dTFIIA-S*, as can the *rolled*^{Sem} mutation expressed from its endogenous enhancer/promoter. These results indicate that the suppression is specific to the activation of the Ras pathway in cone cell precursors and not to any given expression system. The rough-eye phenotype caused by the ectopic expression of the transcription factor *seven-up* (*svp*) in cone cells, *sev-svp2* (Hiromi et al. 1993), is also suppressed by mutations in *dTFIIA-S* (Table 1). As the *sev-svp2* induced eye phenotype is sensitive to Ras pathway signaling levels (Bege- mann et al. 1995), this result is consistent with an effect on components implicated as part of the Ras pathway (or Ras effectors).

Interestingly, other components of the basal transcription machinery tested do not visibly affect the rough-eye phenotype caused by activated Ras pathway components. Deficiencies that remove the 15- and 140-kD polymerase II (PolII) subunits (Parkhurst and Ish-Horowicz 1991; Hamilton et al. 1993) or TBP (Hoey et al. 1990) and point mutants in PolIII40 (Parkhurst and Ish-Horowicz 1991) do not affect the *sev*^{S11} or *sev-ras*^{V12} phenotypes (Table 1; data not shown). This illustrates that reduced levels of other components of the basal transcription machinery, caused by a halving of gene dosage, do not visibly influence the phenotypes of the activated Ras pathway genotypes.

Taken together, these results indicate a specific genetic interaction between Ras pathway and *dTFIIA-S* (and so by implication the holo-TFIIA complex). Furthermore, they suggest that a specific function of TFIIA distinct from its role as a component of the basal transcription complex is responsible for the suppression obtained.

Discussion

Here, we report the dynamic pattern of *dTFIIA-S* expression during *Drosophila* eye development and show that the highest levels of expression coincide with the time window during which Ras pathway activation induces photoreceptor precursor cells. This up-regulation of expression does not depend on the Ras pathway. Although null alleles are cell lethal, hypomorphic *dTFIIA-S* mutations specifically affect the process of photoreceptor development. In addition, full penetrance of the phenotype caused by ectopic activation of the Ras pathway components in cone cells requires both endogenous copies of the *dTFIIA-S* locus. These results indicate that developing photoreceptors are especially sensitive to alterations in the level of *dTFIIA-S* activity and suggest that the in vivo function of *dTFIIA* might be analogous to the activity of *dTFIIA* as an enhancer of transcriptional activation previously observed in vitro (Yokomori et al. 1994).

Developing photoreceptors express high levels of *dTFIIA-S*

In wild-type eyes, the requirement for high levels of TFIIA during photoreceptor determination is met by greatly increased *dTFIIA-S* expression in photoreceptor precursors (Figs. 2E and 3A). Interestingly, the *dTFIIA-S* expression pattern observed in developing photoreceptors precedes the expression of neuronal antigens and is similar in its dynamic regulation to that displayed by the site-specific transcription factors Jun and Pointed (Bohmann et al. 1994; Brunner et al. 1994a; O'Neill et al. 1994). Strikingly, *dTFIIA-S* expression in postgastrulation embryos is also patterned (e.g., increased expression in neuroblasts; Fig. 1B), suggesting that a similar requirement for *dTFIIA-S* during differentiation may also exist in other cell types.

The holo-TFIIA complex has been shown in vitro as being able to both increase the affinity of TBP for the promoter and enhance the potency of proximal transcription factor *trans*-activation domains (Sun et al. 1994; Yokomori et al. 1994). The similarity in expression pattern of Jun, Pointed, and *dTFIIA-S* during photoreceptor induction is striking. Moreover, all three proteins are up-regulated in all photoreceptor precursors and in all cells of the R7 equivalence group that can be transformed to R7 neurons (Dickson et al. 1992a). Thus, it appears possible that the increase in expression of *dTFIIA-S* (one of the components of holo-TFIIA) is required during cellular determination to allow the expression of sufficient levels of effector genes in response to Ras pathway activation. Interestingly, the expression pattern of the large subunit of *dTFIIA* does not appear to be modulated during photoreceptor development. Because biochemical data suggest that *dTFIIA-S* and *dTFIIA-L* are biologically inactive in isolation (Yokomori et al. 1994), *dTFIIA-L* may be present in excess and therefore not be limiting during development. However, further analysis of the *dTFIIA-L* locus is required to confirm this.

Photoreceptor development is sensitive to *dTFIIA-S* levels

In addition to the increased *dTFIIA-S* expression levels present in photoreceptor precursors, phenotypic analysis of mutations in the locus provides further evidence for a role for *dTFIIA-S* in Ras pathway signaling. Because null alleles of *dTFIIA-S* are recessive (not shown), a single copy of the *dTFIIA-S* gene is sufficient for normal eye development. However, a further reduction in *dTFIIA-S* activity in flies homozygous for the hypomorphic allele *dTFIIA-S*^{E73} quite specifically affects photoreceptor development as shown by the disruption in the pattern of R1/R6 and R7 cells in mutant eye imaginal discs (Fig. 4). This phenotype can be rescued by exogenous *dTFIIA-S* expression (Fig. 5B). In addition to the general disruption in ommatidial recruitment, one of the phenotypes observed in both eye imaginal discs and adult clones homozygous for *dTFIIA-S*^{E73} is the presence of extra R7

cells and a reduction in outer photoreceptor number. This suggests a transformation of outer photoreceptors to an inner photoreceptor cell fate (Figs. 4 and 5A). Similar phenotypes are observed in mutations in several genes required for photoreceptor determination, for example, *rough*, *svp*, and *tramtrack* (Tomlinson et al. 1988; Mlodzik et al. 1990; Heberlein et al. 1991; Xiong and Montell 1993). This observation indicates an increased requirement for *dTFIIA-S* during photoreceptor determination.

Like *jun* and *pointed*, *dTFIIA-S* is also expressed at increased levels in other cells of the R7 equivalence group such as the cone cells. These cells are transformed into functional R7 cells by ectopic Ras pathway activation, and the efficiency of this transformation is lowered by reduction of the amount of functional Pointed and Jun proteins (Bohmann et al. 1994; O'Neill et al. 1994). Similarly, when only one copy of *dTFIIA-S* is present, a comparable suppression of cone cell transformation is also observed. This indicates that the level of holo-TFIIA (assumed to be affected by a reduction in *dTFIIA-S* gene dosage) can be a limiting factor during Ras pathway-induced cell fate induction. Thus, a reduction of TFIIA function in cone cell precursors appears to be critical during the Ras-mediated induction.

Concluding remarks

The *in vivo* results presented here are complementary to and extend *in vitro* data showing that holo-TFIIA can act as an enhancer of the *trans*-activation potential of certain site-specific transcription factors (Hori and Carey 1994; Ozer et al. 1994; Sun et al. 1994; Yokomori et al. 1994). A similar function has been attributed to the TAFs (Pugh and Tjian 1990; for review, see Maldonado and Reinberg 1995). This hypothesis is supported further by the recent finding that mutations in some TAFs are also capable of suppressing Ras-mediated, dosage-sensitive phenotypes (D. Wasserman and G. Rubin, pers. comm.). The observed suppression is unlikely to be attributable simply to a reduction in the rate of overall transcription caused by haploinsufficiency of the basal transcription machinery because other components do not show an interaction (Table 1). Thus, the effect is probably caused by a molecular function shared by both TAFs and TFIIA, their coactivator role during activated transcription. In summary, the results presented here suggest that *dTFIIA-S* (and probably holo-TFIIA) is specifically required, in parallel with TAFs, to mediate the interactions of site-specific transcription factors with the basal transcription machinery during activated transcription in development. This may represent an assay with which the specificity of transcriptional activator/basal machinery interactions can be tested in an *in vivo* environment.

Materials and methods

Molecular analysis and generation of *dTFIIA-S* mutations

The *dTFIIA-S* locus was cloned by virtue of its proximity to the

P-element insertion F125. Plasmid rescue DNA was obtained (Mlodzik and Hiromi 1992), and the adjacent transcript was identified and sequenced by standard methods (Sanger et al. 1977; Yokomori et al. 1994).

Mutations in the *dTFIIA-S* locus were generated by the mobilization of the F125 P element after crossing in a chromosome carrying the constitutive $\Delta 2-3$ P-element transposase source (Daniels et al. 1985; Robertson et al. 1988). Resulting stocks were scored for loss of F125 and tested for homozygous lethality. The extent of the resulting genomic deletions was determined by Southern blot analysis. The lethality caused by *dTFIIA-S*^{E73} and the null allele *dTFIIA-S*^{E32} is rescued in transgenic flies carrying constructs, which express *dTFIIA-S*. Rescue constructs contained either the *dTFIIA-S* cDNA expressed from the heat-inducible *hsp70* promoter (pCaSpeR-121) or the 4-kb genomic *EcoRI* fragment containing the transcription unit and P-element insertion site (Fig. 1).

Antibody staining

Antibody stainings were essentially performed as described previously (Tomlinson and Ready 1987). Antibodies against Bar (Higashijima et al. 1992) and β -galactosidase (Promega) were used at 1:300 dilution with 0.3% Triton X-100 and 0.3% Deoxycholate as detergents. The rabbit anti-*dTFIIA-S* serum was preabsorbed against 0- to 12-hr embryos prior to final incubations at 1:2000 dilution, with 0.7% saponin as detergent. Whole-mount staining was carried out using HRP-conjugated secondary antibodies (Bio-Rad). A rat monoclonal antibody against Elav (a kind gift of G. Rubin, University of California, Berkeley) was used for confocal microscopy and Texas Red-conjugated antirabbit IgG and FITC-conjugated antirat IgG (dilution 1:200) secondary antibodies were from Jackson Immuno-research. Scanning confocal images were captured using the EMBL Compact Confocal Microscope.

Generation of mutant clones

Homozygous mutant clones were generated by mitotic recombination events induced either by the FLP/FRT system (Xu and Rubin 1993) or X-ray irradiation (1000 rads) in first-instar larvae. Clones were marked with the *myc* epitope marker present in π M constructs (Xu and Rubin 1993) for analysis in third-instar eye imaginal discs, with the cuticle marker Stubble or the eye color marker *w*⁺ for analysis in adult animals.

Histological analysis of adult retinas

Sections of adult retinæ were made according to Tomlinson and Ready (1987). The average number of R7 cells per ommatidium was scored in apical sections of 5–6 eyes for each genotype shown, and the mean number of R7 photoreceptors per ommatidium was calculated. Experimental and control values of R7/ommatidium were scored in sibling flies. For scanning electron microscopy analysis, heads were dehydrated through an ethanol series, critical point dried, and coated with 20 nm of gold.

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M P Zeidler, K Yokomori, R Tjian, et al.

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