**Drosophila** homologues of the transcriptional coactivation complex subunits TRAP240 and TRAP230 are required for identical processes in eye-antennal disc development

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Accepted 5 December 2000; published on WWW 23 January 2001

**SUMMARY**

We have identified mutations in two genes, blind spot and kohtalo, that encode **Drosophila** homologues of human TRAP240 and TRAP230, components of a large transcriptional coactivation complex homologous to the yeast Mediator complex. Loss of either blind spot or kohtalo has identical effects on the development of the eye-antennal disc. Eye disc cells mutant for either gene can express decapentaplegic and atonal in response to Hedgehog signaling, but they maintain inappropriate expression of these genes and fail to differentiate further. Mutant cells in the antennal disc lose expression of Distal-less and misexpress eyeless, suggesting a partial transformation towards the eye fate. blind spot and kohtalo are not required for cell proliferation or survival, and their absence cannot be rescued by activation of the Hedgehog or Notch signaling pathways. These novel and specific phenotypes suggest that TRAP240 and TRAP230 act in concert to mediate an unknown developmental signal or a combination of signals.

Key words: TRAP240, TRAP230, blind spot, Transcription, Mediator, Drosophila melanogaster, Eye development

**INTRODUCTION**

Development requires the interpretation of specific temporal and spatial signals to yield tightly coordinated patterns of gene transcription. This process has been well studied in the **Drosophila** eye imaginal disc. The eye disc is determined during embryonic and larval stages by the action of the Pax6 transcription factors Eyeless (Ey; Quiring et al., 1994) and Twin of Eyeless (Toy; Czerny et al., 1999) and the downstream transcription factors encoded by eyes absent (eya; Bonini et al., 1993), sine oculis (so; Cheyette et al., 1994; Serikaku et al., 1994) and dachshund (dac; Mardon et al., 1994). Misexpression of these genes in other imaginal discs, particularly in combination, can lead to ectopic eye development (Bonini et al., 1997; Chen et al., 1997; Czerny et al., 1999; Halder et al., 1995; Pignoni et al., 1997; Shen and Mardon, 1997). The antennal disc primordium also expresses ey and toy at embryonic stages (Halder et al., 1998) and is more easily transformed toward the eye fate than other discs (Bonini et al., 1997; Chen et al., 1997; Pignoni et al., 1997).

In the third larval instar a wave of photoreceptor differentiation initiates at the posterior margin of the eye disc and propagates towards the anterior; its front is marked by an indentation in the disc known as the morphogenetic furrow (Ready et al., 1976). Initiation of the morphogenetic furrow requires the secreted factors Hedgehog (Hh) and Decapentaplegic (Dpp), which are expressed at the posterior margin prior to initiation (Borod and Heberlein, 1998; Burke and Basler, 1996; Cho et al., 2000; Dominguez and Hafen, 1997; Royet and Finkelstein, 1997; Wiersdorff et al., 1996). Progression of differentiation also requires both these signals (Chanut and Heberlein, 1997; Heberlein et al., 1993; Ma et al., 1993), although at this stage they are partially redundant. Clones of cells mutant for smoothened (smo), which encodes the Hh receptor, show a partial inhibition of differentiation (Strutt and Mlodzik, 1997), while internal clones of cells mutant for components of the Dpp pathway differentiate almost normally (Burke and Basler, 1996; Wiersdorff et al., 1996). Only in cells unable to respond to either Hh or Dpp is differentiation completely blocked (Curtiss and Mlodzik, 2000; Greenwood and Struhl, 1999). A third signal, Wingless (Wg), is present at the dorsal and ventral margins of the eye disc and prevents ectopic initiation from occurring at those locations (Ma and Moses, 1995; Treisman and Rubin, 1995).

Hh is required for the expression of atonal (ato; Borod and Heberlein, 1998; Dominguez, 1999; Greenwood and Struhl, 1999), which encodes a helix-loop-helix transcription factor (Jarman et al., 1993), in a stripe of cells anterior to the morphogenetic furrow. ato expression is subsequently refined to groups of cells and finally to single R8 photoreceptors (Jarman et al., 1994). Both the initial high-level expression of ato and its refinement require the Notch (N) receptor and its ligand Delta (Dl; Baker et al., 1996; Baker and Yu, 1997; Cagan and Ready, 1989). ato is required for the formation of
R8 photoreceptors and thus indirectly for all photoreceptors (Jarman et al., 1994; Jarman et al., 1995), as R8 recruits the remaining cells in the cluster by producing the ligand Spitz for the EGF receptor (Freeman, 1996; Tio and Moses, 1997). Ato function is antagonized anterior to the furrow by the helix-loop-helix proteins Hairy (H) and Extramacrochaetae (Emc; Brown et al., 1995); interestingly, expression of h in a stripe anterior to the ato domain appears to be directly activated by Dpp and Hh signaling (Greenwood and Struhl, 1999; Heberlein et al., 1995).

Regulation of gene transcription is a complex process requiring not only specific transcriptional activators and repressors, the activity of which may be modified by cell-cell signaling, but also more general factors. Chromatin remodeling complexes are thought to control the accessibility of regulatory sequences to other factors (Kingston and Narlikar, 1999). Another class of mammalian complexes related to the yeast Mediator complex (Kim et al., 1994) has recently been described; such complexes appear to be required as coactivators or in some cases corepressors that link specific factors to the basal transcriptional machinery (Malik and Roeder, 2000). The thyroid hormone receptor-associated protein (TRAP) 25-subunit complex was identified through its ligand-dependent association with the thyroid hormone receptor (Fondell et al., 1996). It appears to be very similar or identical to the SRB and MED-containing cofactor (SMCC) complex isolated by binding to the epitope-tagged SRB10 subunit (Gu et al., 1999; Ito et al., 1999), as well as to the vitamin D receptor interacting protein (DRIP) complex isolated by its association with liganded vitamin D receptor (Rachez et al., 1998; Rachez et al., 1999) and the activator-reruited cofactor (ARC) complex isolated by binding to SREBP (Naar et al., 1999). Other complexes with similar composition include negative regulator of activated transcription (NAT; Sun et al., 1998), murine Mediator (Jiang et al., 1998), human Mediator (Boyer et al., 1999), cofactor required for Sp1 (CRSP; Ryu et al., 1999) and positive cofactor 2 (PC2; Malik et al., 2000). Several of these complexes have been shown to act as coactivators that stimulate transcription in vitro with a variety of activators, including nuclear receptors but also VP16, p53, Sp1, SREBP, E1A and GAL4. The NAT and SMCC complexes can also repress transcription (Gu et al., 1999; Sun et al., 1998).

The roles of the individual subunits of these complexes have mostly not been defined; however, nuclear receptors have been shown to bind directly to TRAP220/DRIP205 (Yuan et al., 1998; Hittelman et al., 1999). Fibroblasts from mice defective for the TRAP220 gene show a reduction in thyroid hormone-stimulated transcription, while the mice have abnormalities of heart and brain development (Ito et al., 2000). The glucocorticoid receptor also binds to DRIP150/DRIP170 through its constitutive AF-1 activation domain (Hittelman et al., 1999). The VP16 and p53 activators bind to TRAP80 (Ito et al., 1999), and E1A interacts with human Sur-2 (Boyer et al., 1999). Interestingly, TRAP240 and TRAP230 are absent from the PC2 and CRSP complexes; as PC2 still exhibits a broad coactivator activity (Malik et al., 2000), no biochemical function has yet been ascribed to these subunits.

We have isolated mutations in the blind spot (bli) and kohtalo (kto) genes, which we have shown to encode homologues of TRAP240 and TRAP230 respectively, in a screen for mutations affecting early eye development in Drosophila. Mutations in the two genes have identical phenotypes; both block photoreceptor differentiation after the initial expression of ato and dpp has been established, and prolong the expression of these genes and others expressed anterior to the morphogenetic furrow. Neither gene is required for growth, and mutant cells do not influence the growth or differentiation of surrounding cells. In the antennal disc, bli and kto are required to prevent the expression of ey and promote the expression of Distal-less (Dll). Cells mutant for bli or kto cannot be induced to differentiate as photoreceptors by activation of the Hh or N signaling pathways. TRAP240 and TRAP230 thus seem to act together to promote specific cell fate decisions, transmitting either a novel signal or a combination of signals.

MATERIALS AND METHODS

Genetics

The mosaic screen for mutations affecting early eye development will be described in detail elsewhere. Briefly, males carrying an FRT site at position 80B were mutagenized with 35 mM EMS and crossed to females carrying the same FRT site, a P(w+) element at position 70C, and FLP recombinase driven by the eyelless (ey) promoter on the X chromosome (eyFLP1; Newsome et al., 2000). Flies were screened in the next generation for scars or head cuticle in the eye in conjunction with the absence of photoreceptors in w- clones. In a screen of 76,230 F1 flies, we identified 19 alleles of kto and 15 alleles of bli. Although these kto alleles fail to complement the lethality of kto- and Df[3L]kto2, the kto- chromosome also carries a second cell-lethal mutation that prevented the recovery of any kto homozygous clones in the eye disc. The transgenic lines used were dpp-lacZ (Blackman et al., 1991), w[+]/Kass1 (Kass1 et al., 1992), arm-lacZ (Vincent et al., 1994), Ubi-GFP (Davis et al., 1995), UAS-hh (Azpiazu et al., 1996), UAS-HACi(m1-4) (Chen et al., 1999), UAS-N[mutation] (Doherty et al., 1996), ey-GAL4 (Hazellett et al., 1998) and UAS-FLP (Duffy et al., 1998). The clones shown were generated using eyFLP, which is active in the antennal disc as well as the eye disc, except those in Figs 2C-D and 3D, which were generated using hsFLP122 induced by a 1 hour heat shock at 38.5°C in both first and second instars, and those in Fig. 5A-F, which were generated using ey-GAL4, UAS-FLP. The ey-GAL4 simultaneously drove the expression of UAS-hh, UAS-HACi(m1-4) or UAS-N[mutation]. The clone in Fig. 2F was made by crossing FRT80, b1[T(1;1)TM6B males to y, w, eyFLP1; FRT80, M(3)67C, P(w+)/TM6B females (Ito and Rubin, 1999).

Immunohistochemistry and in situ hybridization

Third instar larval eye discs were stained with antibodies and X-gal as described (Hazelett et al., 1999). Antibodies used were rat anti-Elav (1:1; Robinow and White, 1991), rabbit anti-Atonal (1:5000; Jarman et al., 1994), rabbit anti-β-galactosidase (1:5000; Cappel), mouse anti-β-galactosidase (1:200; Promega), rat anti-Ci (1:1; Motzny and Holmgren, 1995), mouse anti-H (1:4; Brown et al., 1995), rabbit anti-Ey (1:5000; a gift from Uwe Walldorf), and mouse anti-Dac (1:200; Mardon et al., 1994). Fluorescence images were obtained using a Leica TCS NT confocal microscope. Antisense RNA probes labeled with digoxigenin-UTP homologous to subfragments of the coding regions of bli and kto were used for in situ hybridization; imaginal discs were hybridized as described previously (Maurel-Zaffran and Treisman, 2000).

Molecular biology

Plasmid rescue from the l(3)L7062 line was performed as described (Mlodzik et al., 1990) and DNA adjacent to l(3)R760 was isolated
by inverse PCR. Sequence from these rescued fragments was used to align the P insertions on genomic sequence available from the Berkeley Drosophila genome project (GenBank accession number AC013164). Nearby genomic sequence showed homology to the ESTs GM05325 and LD10285; these were obtained from the genome project and used to screen the LD embryonic cDNA library also obtained from the genome project. The sequence of the longest cDNA obtained has been submitted to GenBank (accession number AF324425). A predicted gene encoding a TRAP230 homologue (CG8491) was identified by searching the Drosophila genome using tblastn. We amplified a 1.5 kb fragment of this gene from wild-type genomic DNA by PCR and used it to screen the LD library. The sequence of the longest cDNA obtained has been submitted to GenBank (accession number AF324426). To identify sequence changes in bli mutant alleles, bli$^{T72}$, bli$^{T860}$ and bli$^{T413}$ were balanced over TM6B, P(Ubi-GFP65T) and homozygous late embryos or first instar larvae were selected by lack of GFP fluorescence and used to prepare genomic DNA. PCR primers were designed to amplify bli exons in fragments of 500 bp-2 kb, which were then sequenced. All observed sequence changes were confirmed on a second independent PCR reaction. The same procedure was followed to identify sequence changes in kto$^{T555}$, kto$^{T24}$ and kto$^{T63}$.

RESULTS

Mutations in blind spot and kohtalo block photoreceptor differentiation

We have undertaken a systematic screen for genes that are required for cells in the Drosophila eye disc to differentiate into retinal tissue, regardless of whether these genes also act at earlier developmental stages (Benlali et al., 2000). The basis of the screen was to use the FLP-FRT system (Xu and Rubin, 1993) to create clones of cells homozygous for a newly induced mutation in the eye of an otherwise heterozygous fly. We used an enhancer from the eyeless (ey) gene to express FLP recombinase specifically in the eye disc (Newcombe et al., 2000). Mosaic flies were screened for scars or head cuticle in the eye in conjunction with the absence of photoreceptors derived from mutant cells marked with white (w). In this way we identified mutations that prevented cells from differentiating as photoreceptors but allowed them to survive long enough to prevent their replacement by surrounding cells.

In our screen on the left arm of the third chromosome, we identified two complementation groups with identical mutant phenotypes (Fig. 1). One of these groups failed to complement the lethality of the trithorax group gene kohtalo (kto; Kennison and Tamkun, 1988); the other is a novel gene that we have named blind spot (bli). We isolated 19 alleles of kto and 15 alleles of bli; at least 10 alleles of each gene were examined by staining larval eye discs with an antibody to the neuronal-specific protein Elav (Robinow and White, 1991) and were found to have indistinguishable phenotypes, suggesting that they are all at least strong loss-of-function alleles.

Clones of cells mutant for either kto or bli contained few or no photoreceptors expressing Elav; when photoreceptors were present they were found in posterior regions of the clone (Fig. 1). Most of these cells apparently die later in development, as scars, rather than w$^+$ clones, were observed in adult eyes (data not shown). Interestingly, the clones appeared to grow to the same size as their wild-type twin spots, and had no effect on the growth of surrounding cells or the shape of the disc as a whole (Figs 1, 2D). Wild-type cells anterior to mutant clones appeared to be at approximately the appropriate stage of development; thus mutant cells did not prevent the spread of differentiation-promoting signals even though they did not themselves respond to these signals. As the bli and kto phenotypes appeared indistinguishable, they will be shown interchangeably in future figures; however, almost all experiments have been carried out using alleles of both genes with identical results.

bli and kto mutant cells initiate differentiation and then arrest

To determine at what stage differentiation was blocked in bli and kto mutants, we examined the expression of atonal (ato), a proneural gene (Jarman et al., 1994) that is activated in the morphogenetic furrow by Hedgehog (Hh) signaling (Dominguez, 1999; Greenwood and Struhl, 1999; Heberlein et al., 1995) and subsequently restricted to the R8 photoreceptors (Jarman et al., 1994). In clones of cells mutant for either bli or kto, expression of ato was weaker than normal in the morphogenetic furrow, but persisted in many of the cells within the clone in regions posterior to the furrow (Fig. 2A,B).
Expression could eventually resolve into single cells, but these were found much further posterior in the disc than in surrounding wild-type tissue.

The expression of decapentaplegic (dpp) was affected in a similar manner. dpp is normally activated by Hh in a stripe in the morphogenetic furrow (Heberlein et al., 1993; Heberlein et al., 1995; Ma et al., 1993; Strutt and Mlodzik, 1997); clones mutant for bli or kto showed reduced dpp-lacZ expression in this stripe, but ectopic expression of dpp-lacZ in posterior regions of the eye disc (Fig. 2C-E). Thus cells mutant for bli or kto appear to respond to Hh by activating the expression of ato and dpp, though at lower levels than wild-type cells, and they fail to terminate the expression of these genes at the normal time.

To test whether Hh signaling was affected in bli and kto mutant clones, we examined the distribution of Cubitus interruptus (Ci). In the absence of Hh signaling the Ci transcription factor is cleaved to a repressor form that is not recognized by an antibody to the C-terminal domain (Azas-Blanc et al., 1997; Motzny and Holmgren, 1995). In bli or kto mutant clones, we found that the upregulation of full-length Ci was weaker than normal in the morphogenetic furrow, but high levels persisted posterior to the furrow (Fig. 2G,H and data not shown). Thus the response to Hh signaling is not terminated at normal time (Dominquez, 1999), so the phenotype could also be due to a lack of late Hh signaling.

To address whether the small number of photoreceptors that form in bli or kto mutant clones resulted from non-autonomous rescue by surrounding wild-type cells, we generated clones in a Minute background (Morata and Ripoll, 1975). This gives the bli or kto mutant cells a growth advantage over cells carrying a Minute mutation; in combination with eyFLP it results in an eye disc entirely composed of mutant tissue (Benali et al., 2000; Bohni et al., 1999; Newson et al., 2000). In such eye discs, some photoreceptors were still able to differentiate, although the size and pattern of clusters were abnormal, and dpp was still expressed (Fig. 2F). The resulting pharate adults had very small eyes composed of bli or kto mutant tissue marked with w (data not shown). Thus a signal from nearby wild-type cells is not necessary for photoreceptors to form from bli or kto mutant cells.

**bli and kto affect genes expressed anterior to the morphogenetic furrow**

Because bli or kto mutant cells seemed to be arrested at an early step in their differentiation, at which they express ato and dpp but not Elav, we examined the effects of these mutations on other genes expressed at various stages of differentiation in the eye disc. The Pax-6 homologue Eyeless (Ey) is normally present only in undifferentiated cells anterior to the morphogenetic furrow at the third instar stage (Halder et al., 1998); however, in bli or kto mutant cells Ey continued to be expressed at high levels posterior to the furrow (Fig. 3A-C). Dachshund (Dac), which is also involved in early steps of eye specification (Chen et al., 1997), is expressed in a broad stripe both anterior and posterior to the morphogenetic furrow, but is down-regulated in more posterior regions of the eye disc (Mardon et al., 1994). Again, bli or kto mutant cells posterior to the normal Dac expression domain maintained high levels of Dac protein (Fig. 3D).

However, not all genes expressed anterior to the furrow were maintained in bli or kto mutant cells. hairy (h) encodes a helix-loop-helix protein that negatively regulates Ato activity and is expressed in a stripe just anterior to the furrow (Brown et al.,...
TRAP240 acts with TRAP230 in the eye disc

1991; Brown et al., 1995; Rushlow et al., 1989); its expression was autonomously reduced in bli or kto mutant cells, and low level expression was maintained only immediately posterior to its normal domain (Fig. 3E-G). This is similar to the effect on h of loss of the Dpp receptor encoded by thick veins (tkv; Greenwood and Struhl, 1999).

Ectopic expression of wingless (wg) can block photoreceptor differentiation and lead to Ey maintenance (Treisman and Rubin, 1995; J. Lee and J. E. T., unpublished data). However, in discs containing clones of cells mutant for bli or kto, wg was still restricted to its normal expression domain at the anterior lateral margins (Fig. 3H).

Antennal disc cell fates are altered in bli and kto mutants

In addition to its maintenance in bli or kto mutant cells in the eye disc posterior to the furrow, we also observed ey misexpression in mutant cells in the antennal disc. However, Ey was not present in every mutant clone and did not always occupy the whole clone (Fig. 4A-D). It is not clear why some mutant cells failed to express ey, as this lack of expression did not appear to correlate with a particular position within the disc. The observed expression of ey suggested that mutant antennal disc cells could be partially transformed towards an eye fate. In agreement with this, expression of the homeobox gene Distal-less (Dll) was lost in bli or kto mutant clones in the antennal disc (Fig. 4E-F); Dll is normally expressed in a broad central domain of the antennal disc but is not found in the eye disc (Cavodeassi et al., 2000; Diaz-Benjumea et al., 1994). In contrast to the non-autonomy observed for ey, Dll expression was autonomously lost in all mutant cells. Although expression of ey in the antennal disc can induce ectopic eyes (Halder et al., 1995), we did not observe these because of the requirement for bli and kto in photoreceptor differentiation. However, mutant clones in the antennal disc did not misexpress ato, and in fact failed to express ato in its normal domain, suggesting that the transformation of antenna to eye was not complete (data not shown).
Another interesting feature of clones in both the antennal disc and the eye disc was that their borders were smooth and rounded, indicating that cells mutant for bli or kto have an altered cell affinity preventing them from mixing normally with wild-type cells (Morata and Lawrence, 1975). This property was observed for clones occurring in several different proximal-distal domains of the antennal disc (Fig. 4 and data not shown).

Hh or N signaling is not sufficient to rescue bli or kto mutants

Because the effects on ato, dpp and Ci suggested that Hh signaling might be altered in bli and kto mutants, we attempted to rescue mutant clones by misexpressing hh throughout the eye disc. However, photoreceptors were still absent from bli or kto mutant clones induced in this background (Fig. 5A and data not shown). To test whether the mutations were blocking Hh signaling downstream of the expression of Hh, we also tried rescuing with an activated form of Ci in which four sites phosphorylated by protein kinase A have been mutated (Chen et al., 1999). However, clones of bli or kto mutant cells generated in eye discs expressing activated Ci still lacked Elav-stained photoreceptors, failed to refine Ato, and maintained Ey expression (Fig. 5B-D and data not shown). Thus the action of bli and kto is epistatic to activation of the Ci protein. The Notch (N) pathway is also important in refining the expression of Ato (Baker et al., 1996) and could be affected by bli and kto. However, expression of the constitutively active intracellular domain of N (Doherty et al., 1996) throughout the eye disc did not alter the misexpression of Ato or the lack of Elav in bli or kto mutant clones (Fig. 5E-F and data not shown).

bli and kto encode homologues of the human TRAP240 and TRAP230 proteins

We identified two P element alleles of bli in the stock center collections, l(3)l7062 and l(3)rK760. Genomic DNA surrounding these P element insertions at 78A2 was isolated by plasmid rescue (for l(3)L7062) or inverse PCR (for l(3)rK760), and its sequence was used to align the insertions on genomic sequence available from the Berkeley Drosophila genome project. Nearby genomic sequence showed homology to several expressed sequence tags (ESTs) that we obtained from the genome project. We then used these ESTs as probes to isolate a full-length 9.8 kb cDNA from an embryonic cDNA library. Both P elements are inserted in the third intron of the corresponding transcript (Fig. 6A). The sequence of the encoded 2618 amino acid protein is shown in Fig. 6C. To confirm that this transcript corresponded to the bli gene, we amplified genomic DNA from several of our EMS-induced alleles of bli and looked for sequence changes. We found changes in three alleles that would introduce stop codons into the predicted open reading frame and would thus be likely to abolish the function of the protein (Fig. 6B). The protein showed two regions of strong homology to the human protein TRAP240 (Fig. 6B,C), which was isolated as part of a large transcriptional coactivation complex that associates with ligand-bound thyroid hormone receptor and vitamin D receptor (Fondell et al., 1996; Ito et al., 1999). The function of TRAP240 within this complex has not been defined.

We used deficiency and recombination mapping to map our kto alleles to 76B1-D5, in agreement with the previously defined map position of the gene (Kennon and Tamkun, 1988). We were unable to find any P elements that failed to complement kto. However, we reasoned that kto was likely to encode another component of the TRAP complex, since its mutant phenotype was identical to that of bli. Searching the Drosophila genome project database for homologues of the human TRAP complex proteins revealed that a predicted TRAP230 homologue, CG8491, mapped to 76D1. We used PCR to amplify a fragment of this predicted gene, which we used as a probe to screen an embryonic cDNA library and isolate full-length 7.7 kb cDNAs. The genomic organization of this transcript is shown in Fig. 7A and the sequence of the

Fig. 5. Activation of the Hh or N pathways does not rescue bli or kto mutant cells. All panels show third instar eye discs from flies with ey-GAL4 and (A) UAS-hh; (B-D) UAS-ci(m1-4); (E,F) UAS-N\textsuperscript{intr}. A and B show anti-Elav staining in brown; kto\textsuperscript{T843} clones (A) and bli\textsuperscript{T606} clones (B) are marked by the lack of blue X-gal staining reflecting arm-lacZ expression. Photoreceptors are absent from bli or kto clones even in the presence of ectopic Hh or activated Ci. (C-F) show anti-Ato staining in green; bli\textsuperscript{T606} clones are marked by the lack of anti-\beta-galactosidase staining reflecting arm-lacZ expression (red in D); (E,F) bli\textsuperscript{T843} clones are marked by the lack of anti-\beta-galactosidase staining reflecting arm-lacZ expression (red in F). Ato expression persists in posterior bli or kto mutant clones even in the presence of activated Ci or activated N.
**Fig. 6.** bli encodes a TRAP240 homologue. (A) Genomic organization of the bli locus. Exons are shown as boxes with the translated regions filled in. The two P element insertions causing bli mutations are shown as inverted triangles at their insertion positions in the third intron. (B) Diagram of the Bli protein. The blocks of homology to TRAP240 are shaded. The positions of the mutations in bli^T606, bli^T13 and bli^F113 are shown. (C) Amino acid sequence of the Bli protein (fly) with homologous regions of TRAP240 (human) aligned below. Identical amino acids appear white on a black background, and similar amino acids appear white on a gray background. Only regions identified as homologous by the blastp program are shown.
Fig. 7. \textit{kto} encodes a TRAP230 homologue. (A) Genomic organization of the \textit{kto} locus. Exons are shown as boxes with the translated regions filled in. (B) Diagram of the \textit{Kto} protein. The region of homology to TRAP240 is shaded. The positions of the mutations in \textit{kto}^T555, \textit{kto}^T241 and \textit{kto}^T637 are shown. (C) Amino acid sequence of the \textit{Kto} protein (fly) with homologous regions of TRAP230 (human) aligned below. Identical amino acids appear white on a black background, and similar amino acids appear white on a gray background. Only regions identified as homologous by the blastp program are shown.
encoded 2531 amino acid protein in Fig. 7C. Homology to TRAP230 is predominantly in the N-terminal two-thirds of the protein, with the remainder of the protein being glutamine-rich in both species (Fig. 7B,C; Ito et al., 1999). We sequenced genomic DNA corresponding to the TRAP230 transcript amplified from several of our kto mutant alleles, and again found changes in three alleles that would cause premature termination of the TRAP230 protein (Fig. 7B). Thus the kto mutant phenotype is likely to result from disruption of TRAP230. We used in situ hybridization to show that both TRAP230 and TRAP240 transcripts were present throughout the eye disc and other imaginal discs (data not shown). This is in agreement with the mutant phenotypes seen when bli or kto function is removed from cells at any location in the eye-antennal disc.

DISCUSSION

Functions of TRAP240 and TRAP230 in the TRAP complex

We have identified two Drosophila homologues of components of the TRAP/DRIP complex based on their requirement for photoreceptor differentiation. The very specific mutant phenotypes of these genes offer a new avenue for understanding the roles of the encoded proteins in transcriptional regulation. The TRAP complex and complexes related to it have been isolated by several different procedures. TRAP240 and TRAP230 are present in the complexes isolated by binding to liganded thyroid hormone receptor, liganded vitamin D receptor, steroid response element binding protein (SREBP), VP16, the p65 subunit of NF-κB, or tagged Srb10 (Fondell et al., 1996; Gu et al., 1999; Ito et al., 1999; Naar et al., 1999; Rachez et al., 1998). However, they are not present in the Sp1 cofactor CRSP (Ryu et al., 1999) or in the PC2 coactivation factor isolated from the USA fraction, which contains many other components of the TRAP complex (Malik et al., 2000). PC2 is sufficient for the general transcriptional coactivation functions previously ascribed to the TRAP complex (Malik et al., 2000); thus TRAP240 and TRAP230 cannot be essential for activation. Our finding that they are required for specific developmental processes in the Drosophila eye, but not for cell growth or viability, suggests that they are not core components required for all activities of the complex. Thus their presence or absence in individual mediator-related complexes may reflect real differences in activity rather than being an artefact of the purification method used. Mutations in kto were previously isolated as suppressors of the Polycomb phenotype, making it a member of the trithorax group of genes (Kennison and Tamkun, 1988). This is consistent with a general role in transcription, as other members of this group have been shown to encode components of a chromatin remodeling complex (Papoulas et al., 1998; Crosby et al., 1999; Collins et al., 1999).

TRAP220, which is not consistently present in PC2 (Malik et al., 2000), has been shown to bind directly to the thyroid hormone receptor and other nuclear receptors (Hittelman et al., 1999; Yuan et al., 1998), and is thought to link these to the coactivation complex; the mouse homologue is required for cells to respond normally to thyroid hormone (Ito et al., 2000). It seems unlikely that the nuclear receptor for the steroid hormone ecdysone is the target of bli and kto; loss of ecdysone receptor function does not prevent photoreceptor differentiation (Brennan et al., 2001), and loss of the coreceptor encoded by ultraspicae causes an acceleration of the morphogenetic furrow (Zelhof et al., 1997). Although there are many other potential nuclear receptors in the genome for which the phenotype in the eye is not known, the interaction of human nuclear receptors with the TRAP complex seems to be specifically through TRAP220 (Yuan et al., 1998). As the Drosophila homologue of TRAP220 also maps to the left arm of the third chromosome, we would have expected to identify mutations in it in our screen if they produced the same phenotype as bli and kto. However, we did not find any other complementation groups with this phenotype. Thus one possibility is that TRAP240 and TRAP230 serve a similar function for an unidentified transcription factor. Alternatively, they may regulate the activity of the TRAP complex.

The identical mutant phenotypes we observe for bli and kto strongly suggest that TRAP240 and TRAP230 act as an obligate heterodimer. Interestingly, homologues of TRAP240 and TRAP230 are present in the C. elegans genomic sequence, while homologues of several other components of the TRAP complex, including TRAP220, TRAP100 and TRAP95, are apparently absent; the functions of TRAP240 and TRAP230 may thus be more ancient than that of TRAP220. Mutations in the C. elegans TRAP230 homologue, sop-1, have recently been shown to act as recessive suppressors of a mutation in an enhancer required for the transcription of the pal-1 gene (Zhang and Emmons, 2000). However, sop-1 mutations and RNA interference with sop-1 have no phenotype in animals wild type for pal-1 (Zhang and Emmons, 2000), suggesting either that TRAP230 activity is partially redundant in C. elegans, or that the function of TRAP230 was not completely abolished. C. elegans homologues of the MED6, MED7 and MED10 proteins also found in PC2 have been shown to be required for embryonic survival and the expression of two genes activated early in embryogenesis (Kwon et al., 1999).

Functions of bli and kto in eye development

The phenotypes caused by bli or kto mutations do not correspond exactly to those due to inhibition or activation of any signaling pathway known to act in eye development. Some aspects of the bli and kto phenotypes resemble defects in Hh or N signaling. Loss of the response to Hh signaling, for instance, in clones lacking the Hh receptor Smoothened (Smo), prevents ato and dpp expression (Domínguez, 1999; Greenwood and Struhl, 1999; Strutt and Mlodzik, 1997); although these responses are diminished in bli and kto mutants they also persist posterior to the morphogenetic furrow, where cells are normally unresponsive to Hh. The basis for this Hh insensitivity is unknown; it could directly require the functions of bli and kto, or it could be a downstream effect of differentiation, which does not occur in bli or kto mutants. Supporting the second model, cells mutant for N also fail to differentiate and continue to express ato in all cells posterior to its normal expression domain (Baker et al., 1996; Baker and Yu, 1997). Like bli or kto mutant cells, they also maintain high levels of full-length Ci (Baker and Yu, 1997). This may indicate that the Hh pathway remains active; however, it has also been suggested that Hh acts at short range to down-
regulate Ci and terminate ato expression (Domínguez, 1999). The ability of the Hh signal to pass through large bli or kto clones and trigger anterior cells to differentiate suggests that the mutant cells may be able to express hh and relay the signal.

The Hh and N signaling pathways are both required for normal growth of cells in the eye disc (Cho and Choi, 1998; Domínguez and de Celis, 1998; Heberlein et al., 1995; Kurata et al., 2000; Ma et al., 1993; Pan and Rubin, 1995; Papayannopoulos et al., 1998; Strutt et al., 1995); mutations in bli or kto appear to have no effect on growth, arguing that they do not mediate all the functions of Hh or N. We were also unable to induce photoreceptor differentiation in bli or kto mutant cells by activating the Hh or N pathways. However, this cannot be taken as definitive evidence that TRAP240 and TRAP230 do not influence signaling through these pathways; if they act as transcriptional coactivators, they may be required for the activated forms of Ci and N that we used to function effectively.

The bli and kto mutant phenotypes also share some features with misregulation of other signaling pathways. Loss of the response to Dpp signaling, like loss of bli or kto, prevents expression of h and leads to prolonged expression of ey (Curtiss and Mlodzik, 2000; Greenwood and Struhl, 1999). However, the phenotypes differ in that cells mutant for components of the Dpp pathway, but not for bli or kto, can differentiate normally if they are not at the posterior margin (Burke and Basler, 1996; Wiersdorff et al., 1996), and loss of Dpp signaling interferes with cell growth and survival (Burke and Basler, 1996). Ectopic activation of the Wg pathway blocks photoreceptor differentiation, but it also promotes proliferation and does not cause persistent dpp expression (Theisen et al., 1996; Treisman and Rubin, 1995). EGF receptor signaling is unlikely to be blocked by bli or kto mutations, as it is strongly required for cell proliferation and survival, but not for the refinement of Ato into single cells or the expression of Elav in those cells (Domínguez et al., 1998; Kumar et al., 1998; Lesokhin et al., 1999). It is possible that bli and kto could antagonize the function of the homeodomain protein Homothorax (Hth), which has been shown to be capable of blocking photoreceptor formation downstream of dpp expression (Pai et al., 1998; Pichaud and Casares, 2000). However, hth is normally restricted to the anterior margin and was not ectopically expressed in bli or kto clones (data not shown).

The presence of small numbers of photoreceptors in clones mutant for null alleles of bli or kto shows that these genes are not absolutely required for photoreceptors to differentiate. As photoreceptors form even in clones that occupy the whole eye disc, their presence cannot be due to rescue by a signal from surrounding wild-type cells. Instead it seems that the process requiring bli and kto function can be autonomously bypassed in a small number of cells.

**Other developmental functions of bli and kto**

In the antennal disc, Dil expression is lost in all cells of bli or kto mutant clones, but ey misexpression is observed only in a subset of mutant cells; we do not know whether this is due to rescue by surrounding wild-type cells. Dil is thought to be activated in response to the combined signaling activity of Dpp and Wg (Lecuit and Cohen, 1997) and to be repressed by Iroquois complex genes in the dorsal eye disc (Cavodeassi et al., 2000). It is not clear which of these regulators might be affected by bli and kto. It is also not known what normally prevents ey expression in the antennal disc, except that this block can be overcome by the ectopic expression of toy, eya, dac, optix or tsb (Bonini et al., 1997; Chen et al., 1997; Czerny et al., 1999; Pan and Rubin, 1998; Pignoni et al., 1997; Seimiya and Gehring, 2000; Shen and Mardon, 1997). bli or kto mutant clones in the wing disc did not misexpress ey (data not shown), suggesting that its activation requires factors specific to the antennal disc as well as the absence of bli and kto. One possibility is that normal ey expression in the embryonic antennal disc (Halder et al., 1998) is inappropriately maintained in bli or kto mutant cells, which could then be considered arrested at an early stage of their development like cells in the eye disc.

A further interesting feature of bli and kto mutant clones is their apparent alteration in cell affinity, shown by the smooth borders formed with surrounding cells. Such borders can form as a result of changes in proximal-distal (P-D) identity in the leg disc (Wu and Cohen, 1999), although the same genes do not appear to control this property in the antennal disc (Casas and Mann, 1998; Dong et al., 2000). It is not clear whether altered P-D identity is the basis for the failure of bli or kto mutant cells to mix normally, as we have not found any marker of P-D identity to be misexpressed in these cells (data not shown).

While this paper was under review, Boube et al. (Boube et al., 2000) also described mutations in the Drosophila homologue of TRAP240, using the name poils aux pattes (pap). They found that a deletion of the first 23 amino acids of TRAP240 caused a distal to proximal transformation of mutant cells in the tarsus of the first leg, but it did not affect the development of the antenna or wing. In contrast, all the alleles we have analyzed cause specific cell fate changes in the antennal and wing discs leading to pronounced malformations in the adult (Fig. 4 and data not shown). The reason for this difference is unclear.

The lack of previously described mutations with the same phenotypes as bli and kto suggests several possible models for their function. TRAP240 and TRAP230 could be required to link a novel transcription factor to the TRAP coactivation complex. They could be required for some, but not all, of the functions of one or several known transcription factors acting in the pathways discussed above. Or they could themselves be transcription factors that were copurified with the TRAP complex by virtue of their strong interactions with it. In this case, their ubiquitous expression would suggest that their activity is modified in some way to produce specific patterns of expression of their target genes. Further biochemical and genetic analysis of the functions of the TRAP proteins will be required to distinguish these alternatives.

We thank Sean Carroll, Barry Dickson, Manfred Frasch, Ulrike Heberlein, Robert Holmgren, Yuh-Nung Jan, Armen Manoukian, Graeme Mardon, Sarah Smolik, Uwe Waldorf, the Bloomington Drosophila stock center and the Berkeley Drosophila genome project for fly stocks and reagents. We are grateful to Florence Janody for in situ hybridization with kto and to Aude Benlali, Ian Oliver and Zara Martirosyan for technical assistance. We thank Ruth Lehmann and Michelle Starz-Gaiano for help with confocal microscopy. The manuscript was improved by the critical comments of Russ Collins, Florence Janody, Jeff Lee and Corinne Zaffran. This work was
supported by grants from the National Institutes of Health (GM56131) and the National Science Foundation (IBN-9728140).

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interact to direct the pattern of retinal differentiation in the eye disc.


TRAP240 acts with TRAP230 in the eye disc


