A Mosaic Genetic Screen Reveals Distinct Roles for *trithorax* and *Polycomb* Group Genes in Drosophila Eye Development

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ABSTRACT

The wave of differentiation that traverses the Drosophila eye disc requires rapid transitions in gene expression that are controlled by a number of signaling molecules also required in other developmental processes. We have used a mosaic genetic screen to systematically identify autosomal genes required for the normal pattern of photoreceptor differentiation, independent of their requirements for viability. In addition to genes known to be important for eye development and to known and novel components of the Hedgehog, Decapentaplegic, Wingless, Epidermal growth factor receptor, and Notch signaling pathways, we identified several members of the *Polycomb* and *trithorax* classes of genes encoding general transcriptional regulators. Mutations in these genes disrupt the transitions between zones along the anterior-posterior axis of the eye disc that express different combinations of transcription factors. Different *trithorax* group genes have very different mutant phenotypes, indicating that target genes differ in their requirements for chromatin remodeling, histone modification, and coactivation factors.

THE Drosophila eye is an excellent model system in which to study developmental processes such as specification of a tissue, propagation of a signal, or cell-cell interactions leading to cell fate determination. The eye imaginal disc is formed in the embryo and specified in the second larval instar by a hierarchy of transcription factors: the two Pax-6 homologs Twin of Eyeless (Toy) and Eyeless (Ey), the compound transcription factor formed by Eyes absent (Eya) and the homeodomain protein Sine oculis (So), and the Ski-related protein Dachshund (Dac; Bonini *et al.* 1993; Mardon *et al.* 1994; Quiring *et al.* 1994; Chen *et al.* 1997; Pignoni *et al.* 1997; Halder *et al.* 1998; Czerny *et al.* 1999). In the third instar, a wave of photoreceptor differentiation marked by an indentation called the morphogenetic furrow propagates across the eye disc. This wave is driven by Hedgehog (Hh), a secreted protein first expressed at the posterior margin of the disc and subsequently in the photoreceptors (Ready *et al.* 1976; Heberlein *et al.* 1993; Ma *et al.* 1993). The eye disc differs from the wing and leg discs in that Hh indirectly activates its own expression in target cells, causing a dynamic expansion of the Hh expression domain. When cells differentiate as photoreceptors, they are no longer able to respond to Hh; thus Hh target genes are only transiently activated and must undergo rapid changes in their expression state (Schwartz *et al.* 1995; Dominguez *et al.* 1996; Strutt and Mlodzik 1996).

One target of Hh is *atonal* (*ato*), a proneural gene responsible for specifying the first photoreceptor, R8 (Jarman *et al.* 1994; Dominguez 1999). The Notch pathway also contributes both to the initial activation of *ato* and to its restriction to a single cell (Cagan and Ready 1989; Baker and Yu 1997; Baonza and Freeman 2001). Once R8 has been determined, it recruits the remaining seven photoreceptors and four cone cells to the ommatidium by secreting Spitz, a ligand for the epidermal growth factor receptor (EGFR; Freeman 1996; Tio and Moses 1997). Another gene activated by Hh is *decapentaplegic* (*dpp*), which encodes a bone morphogenetic protein-related signaling molecule (Heberlein *et al.* 1993; Ma *et al.* 1993). Dpp activates the expression of *hairy* (*h*), an inhibitor of Atonal function expressed in a region anterior to the morphogenetic furrow, known as the preproneural zone (Greenwood and Struhl 1999). In addition, Dpp represses *homothorax* (*hth*), which encodes a homeodomain protein that acts in concert with Ey and the zinc-finger protein Teashirt (Tsh) to repress expression of Eya, So, and Dac in the anterior region of the eye disc (Bessa *et al.* 2002). Dpp thus indirectly allows expression of these molecules in the preproneural zone. Marginal regions of the eye disc contribute to head cuticle rather than to the eye itself; their development is controlled by *wingless* (*wg*), which antagonizes photoreceptor differentiation (Ma and Moses 1995; Treisman and Rubin 1995; Royet and Finkelstein 1997).

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Sequence-specific transcription factors play a critical role in directing the expression of their target genes; however, transcription is also regulated by more general factors that control chromatin structure or recruitment of the basal transcription machinery. Genes of the *Polycomb* group encode proteins that contribute to the repression of homeotic and other genes (Orlando 2003). Two major complexes of *Polycomb* group proteins have been identified. One contains Enhancer of zeste [E(z)], a SET domain protein that has recently been shown to have histone methyltransferase activity, with a preference for lysine 27 of histone H3 (Cao *et al.* 2002; Czermin *et al.* 2002; Kuzmichev *et al.* 2002; Muller *et al.* 2002). The second complex, Polycomb repressive complex 1 (PRC1), can block nucleosome remodeling by SWI/SNF-related complexes (Francis *et al.* 2001). PRC1 contains Polycomb (Pc), a chromodomain protein, which binds to H3 methylated at K27 (Cao *et al.* 2002; Fischle *et al.* 2003; Min *et al.* 2003), allowing the complex to be recruited by E(z) activity. The complex also includes Polyhomeotic (Ph), Posterior sex combs (Psc), Sex combs on midleg (Scm), and dRING1, which have not yet been assigned enzymatic activities.

Genes of the *trithorax* group were identified as suppressors of *Polycomb* phenotypes and have therefore been implicated in activation of homeotic genes (Kennison and Tamkun 1988; Simon 1995); however, their functions are quite heterogeneous. Three members of this group, *brahma* (*brm*), *moira* (*mor*), and *osa*, encode components of the SWI/SNF-related Brahma chromatin-remodeling complex (Tamkun *et al.* 1992; Papoulas *et al.* 1998; Collins *et al.* 1999; Crosby *et al.* 1999). This complex alters the positions of nucleosomes or their interactions with DNA to modulate transcription factor accessibility (Narlikar *et al.* 2002) and is likely to be involved in gene repression as well as activation (Holstege *et al.* 1998; Collins *et al.* 1999; Collins and Treisman 2000; Martens and Winston 2002). Two other *trithorax* group genes, *skuld* (*skd*) and *kohtalo* (*kto*), encode subunits of an accessory submodule of the Drosophila mediator complex (Boube *et al.* 2000, 2002; Treisman 2001; Janody *et al.* 2003). The mediator complex is required even in the absence of nucleosomes to link transcriptional activators or sometimes repressors to the basal transcriptional machinery (Rachez and Freedman 2001). Homologs of the Trithorax (Trx) protein, and probably also Trx itself, act as histone methyltransferases for lysine 4 of H3 (Roguev *et al.* 2001; Czermin *et al.* 2002; Milne *et al.* 2002; Nagy *et al.* 2002; Nakamura *et al.* 2002); unlike K9 and K27, methylation of K4 is associated with transcriptional activation (Wang *et al.* 2001; Santos-Rosa *et al.* 2002; Ng *et al.* 2003). Another SET domain *trithorax* group protein, Absent, small and homeotic discs 1 (Ash1), similarly methylates K4 and K9 of H3, as well as K20 of H4, and appears to recruit the Brm complex (Beisel *et al.* 2002). The function of Ash2, a PHD protein, is not known, although its yeast homolog Bre2 associates with a Trx homolog, Set1 (Adamson and Shearn 1996; Roguev *et al.* 2001). Other members of the group include *kismet*, which encodes several large chromodomain proteins (Daubresse *et al.* 1999; Therrien *et al.* 2000), *Trithorax-like*, which encodes GAGA factor (Farkas *et al.* 1994), and additional, less well-characterized genes (Kennison and Tamkun 1988; Gildea *et al.* 2000; Calgaro *et al.* 2002; Gutierrez *et al.* 2003).

Almost all genes known to act in eye development are also required for embryonic survival; thus additional genes important for eye development may remain unidentified because they cause early lethality when mutated. Establishment of the FLP-FLP recognition target (FRT) system for mitotic recombination in Drosophila (Xu and Rubin 1993) and use of FLP recombinase driven by the eye-specific *ey* enhancer to promote recombination at high efficiency in the eye disc (Newsome *et al.* 2000) have made it possible to systematically identify genes required for photoreceptor differentiation. A mosaic screen of this nature should also allow identification of novel components of the Hh, Dpp, Wg, and EGFR signaling pathways; those that are maternally contributed to the embryo and required for oogenesis would be particularly difficult to find by other methods. We have used this technique to screen the autosomes for genes required for normal photoreceptor differentiation. In addition to known and novel components of the signaling pathways that contribute to patterning the eye disc, we identified several *trithorax* and *Polycomb* group genes. The markedly different mutant phenotypes of different members of the *trithorax* group suggest that they are used to regulate different target genes *in vivo*. The rapid gene expression transitions that occur during morphogenetic furrow progression may require a variety of transcriptional control mechanisms.

MATERIALS AND METHODS

Fly stocks and genetics: For the screen, *w* flies carrying the FRT40, FRT42, FRT80, or FRT82 insertions were isogenized for the corresponding chromosome; males were then mutagenized with 25–35 mm ethyl methanesulfonate (EMS) and crossed to *y*, *w*, *ey*FLP1; FRT40 (or 42, 80, or 82), P(w^+, *armlacZ*) females. Flies were allowed to lay eggs for 5 days and then discarded. F_1 progeny were screened for reduced eyes containing no visible white tissue. Such flies were mated to the appropriate balancer stock (*w*; *CyO/Sco* or *w*; *TM3/TM6B*). In the next generation, three *white* males were mated individually to *y*, *w*, *ey*FLP1; FRT40, P(w^+, *armlacZ*)/*CyO* (or the analogous stock for the other chromosome arms). If the reduced-eye phenotype was observed in flies carrying both FRT chromosomes, the balancer flies were used to generate a stock carrying the mutant chromosome. Complementation tests were performed with alleles of the following candidate genes: *smoothened*, *thick veins*, *dpp*, *Mothers against dpp*, *wg*, *Protein kinase A*, *spitz*, *Star*, *son of sevenless*, *eya*, *dac* (2L); *patched*, *costal-2*, *tout velu*, *Epidermal growth factor receptor*, *downstream of receptor kinases*, *leonardo*, *so* (2R); *vein*, *daughter of sevenless*, *fringe*, *eyegone*, *naked* (3L); *supernumerary limbs*, *punt*, *Medea*, *pointed*, *ras1*, *ato*, *glass*

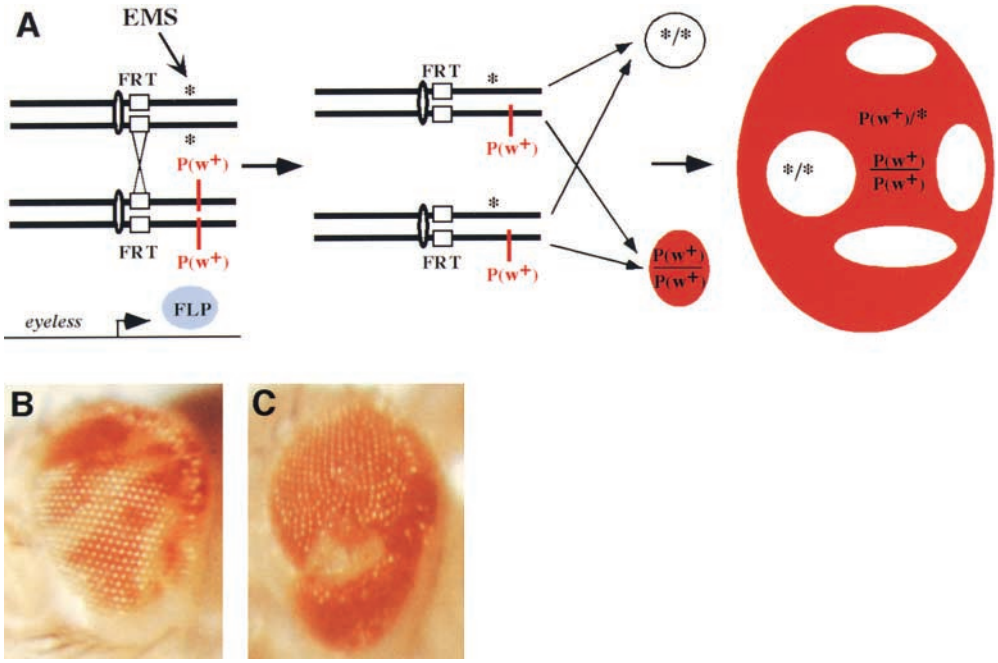

FIGURE 1.—Design of the screen. (A) *ey*FLP was used to induce exchange of a chromosome arm between chromatids on homologous chromosomes after replication specifically in the eye-antennal disc. Separation of the chromatids at mitosis produced one daughter cell homozygous for the chromosome arm carrying an EMS-induced mutation. These mutant cells lost the P(w^+) element on the wild-type chromosome arm and thus produced *white* clones in the adult eye. (B and C) Adult eyes with clones generated as described in A. (B) Phenotypically normal *white* clones. (C) shn^{56M} clones, which produce scars in the eye with no visible *white* tissue. Mutations like this were selected in the screen.

(3R). The remaining complementation groups were first mapped by crossing to the Bloomington deficiency kit for the appropriate chromosome arm. Testing likely genes in a region defined in this way allowed us to identify additional groups as *lines*, *arrow* (*arr*), *hyperplastic discs*, *schnurri* (*shn*), *axin*, *kuzbanian*, *nicastrin*, *scribbled* (*scrib*), *brm*, *trx*, *E(z)*, *Pc*, and *belle*. Our *connector enhancer of ksr* mutations complemented the entire deficiency kit, but were identified on the basis of a phenotype resembling that of EGFR pathway mutations and were confirmed by complementation testing. Further fine-scale mapping and cloning were required to identify *sightless* (LEE and TREISMAN 2001), *Myosin binding subunit*, *act up* (BENLALI *et al.* 2000), *kto*, and *skd* (TREISMAN 2001).

The alleles of *arr*, *lines*, *shn*, *scrib*, *skd*, *kto*, *brm*, *E(z)*, and *Pc* used here were identified in the above screen. Several of our alleles of *skd* and *kto* have been shown to introduce early stop codons (TREISMAN 2001). Pc^{T181} was sequenced and shown to change K92 to a stop codon, truncating the protein shortly after the chromodomain. $E(z)^{E4G.2}$ was sequenced and shown to change R622 to a stop codon, truncating the protein at the beginning of the SET domain. Thus these are likely null alleles. The other alleles we isolated have not been sequenced. The null alleles trx^{E2} and mor^{1} are described in GINDHART and KAUFMAN (1995), and osa^{308} is described in TREISMAN *et al.* (1997). Other stocks used were *dpp-lacZ* (BLACKMAN *et al.* 1991), UAS-*Ubx* (CASTELLI-GAIR *et al.* 1994); *y*, *w*, *ey*FLP1; FRT82, *M(3)96C*, *arm-lacZ*; *y*, *w*, *ey*FLP1; FRT82, $RpS3^{1}$, *Ubi-GFP*; *y*, *w*, *ey*FLP1; FRT80, *M(3)67C*, *Ubi-GFP*; *y*, *w*, *ey*FLP1; FRT82, *Ubi-GFP*; and *y*, *w*, *ey*FLP1; FRT80, *Ubi-GFP*. To generate Ubx-expressing clones, FRT82, UAS-*Ubx* males were crossed to *ey*FLP, UAS-*GFP*; *tub*-GAL4, FRT82, *tub*-GAL80/TM6B females.

Immunohistochemistry: Third instar eye discs were stained as described (HAZELETT *et al.* 1998). Antibodies used were rat anti-Elav (1:5; ROBINOW and WHITE 1991), rabbit anti-β-galactosidase (Cappel, 1:5000), rabbit anti-Hth (1:500; KURANT *et al.* 1998), rabbit anti-Tsh (1:2000; WU and COHEN 2000), rabbit anti-Ey (1:1000; HALDER *et al.* 1998), mouse anti-Eya (1:1; BONINI *et al.* 1993), mouse anti-Dac (1:200; MARDON *et al.* 1994), mouse anti-H (1:5; CARROLL *et al.* 1988), and mouse anti-Ubx (1:10; WHITE and WILCOX 1984). Fluorescent images were obtained using a Leica TCS NT confocal microscope.

RESULTS

A mosaic genetic screen for genes required for photoreceptor differentiation: Most of the genes previously shown to play a role in early eye development were identified either through eye-specific alleles or by testing the function in the eye of genes known to be required for embryogenesis. To systematically identify genes controlling the pattern of photoreceptor differentiation only on the basis of their phenotype in the eye and regardless of earlier requirements for viability, we used a mosaic approach (XU and RUBIN 1993). Male flies isogenic for a chromosome carrying an FRT insertion close to the centromere were mutagenized with ethyl methanesulfonate (EMS) and mated to females that had both the same FRT linked to a *P* element carrying the *white* gene [P(w^+)] and FLP recombinase driven by the eye-specific *eyeless* enhancer (*ey*FLP; NEWSOME *et al.* 2000; Figure 1A). This resulted in F_1 progeny with clones in the eye that were homozygous for mutations on the chromosome arm carrying the FRT and lacked w^+ expression (Figure 1B). Adult flies were screened for reductions in eye size or scars in the eye accompanied by a lack of *white* tissue, indicating clones that failed to differentiate into photoreceptors but persisted long enough to prevent their replacement by wild-type cells (Figure 1C). Mutant chromosomes were balanced and retested by crossing to the *ey*FLP stock. As a secondary screen, mutant clones in the third instar eye disc were stained for the neuron-specific nuclear protein Elav (ROBINOW and WHITE 1991), and wild-type tissue was marked by X-gal staining of an *armadillo* (*arm*)-*lacZ* insertion present on the nonmutagenized chromosome (VINCENT *et al.* 1994). Mutations were discarded if the clones appeared wild type at this stage or if cell prolifera-

TABLE 1

Genes identified in the screen

Function	No. of alleles[a]
Hh pathway	
patched	10
costal-2	4
lines	2
supernumerary limbs	6
smoothened	1
sightless	2
hyperplastic discs	4
Dpp pathway	
thick veins	2
decapentaplegic	2
punt	7
Mothers against dpp	4
Medea	5
schnurri	9
Wg pathway	
supernumerary limbs	6
axin	1
arrow	2
lines	2
EGFR pathway	
Star	9
pointed	10
Epidermal growth factor receptor	6
downstream of receptor kinases	3
son of sevenless	2
daughter of sevenless	1
ras1	2
leonardo	1
connector enhancer of ksr	3
Notch pathway	
fringe	3
kuzbanian	4
nicastrin	1
Eye-specification genes	
eyes absent	5
dachshund	1
sine oculis	5
Other candidate genes	
atonal	5
glass	5
Cytoskeletal genes	
Myosin binding subunit	4
act up	2
scribbled	4
trithorax/Polycomb group genes	
kohtalo	19
skuld	15
brahma	6
trithorax	1
Enhancer of zeste	2
Polycomb	3
belle (RNA-binding protein)	2
Unidentified genes	
Group A	10
Group B	9
Groups C, D	6
Groups E, F, G	4
Groups H, I, J	3
Groups K, L, M, N, O, P	2
Single hits	52

[a] The number of alleles isolated for each gene.

tion within the clone was severely reduced. Mutations showing defects in the pattern of photoreceptor differentiation were placed into complementation groups and tested for their ability to complement candidate genes on the same arm that were known to affect eye development (see MATERIALS AND METHODS).

All four major autosomal chromosome arms, 2L, 2R, 3L, and 3R, were screened in this way. A total of 302,040 F_1 flies were screened, ranging from 45,000 to 104,000 per chromosome arm. We found 2559 mutants, of which 1391 were fertile. We were able to recover and balance 613 of these; loss at this stage could have been due to mosaicism of the F_1 mutants or to recombination between the mutation and the P(w^+) element in female mutants. Following the secondary screen, 301 lines were retained. Their distribution between known genes, unidentified complementation groups, and single hits is given in Table 1. We identified most of the expected genes, including many components of the Hh, Dpp, Wg, and EGFR pathways. Two novel regulators of Hh signaling, *sightless* and *hyperplastic discs*, were isolated in this screen (LEE and TREISMAN 2001; LEE *et al.* 2002). Some components of these pathways may have been missed because they act nonautonomously (*e.g.*, *hh*, *dispatched*, and *spitz*), are redundant (*e.g.*, *rhomboid* and *roughoid*), are on the X or fourth chromosome or are proximal to the FRT used (*e.g.*, *shaggy*, *cubitus interruptus*, and *rolled*), or are encoded by small genes. A pilot screen that would have allowed identification of nonautonomous mutations because clones were generated in a *Minute* background (MORATA and RIPOLL 1975) was unsuccessful due to high levels of lethality, presumably caused by leaky *ey*FLP expression in other tissues. We note that although *arrow* and *lines* are both required to mediate Wg signaling in the embryo (HATINI *et al.* 2000; WEHRLI *et al.* 2000), their phenotypes in the eye were distinct. Unlike *dishevelled* and *arrow*, *lines* did not induce

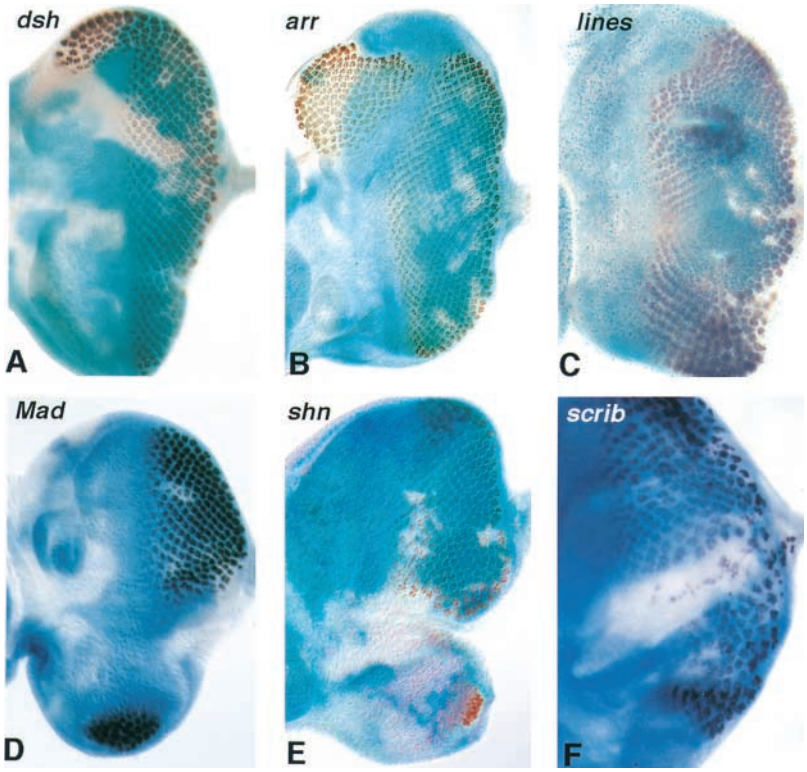

Figure 2.—Phenotypes of known genes identified in the screen. In this and subsequent figures third instar eye discs are shown with posterior to the right. Photoreceptors are stained with Elav in brown in A–F, and blue X-gal staining marks wild-type tissue expressing *arm-lacZ*. (A) dsh^{v26} clones. (B) arr^{63D} clones. (C) $lines^{13B}$ clones. While *arr* and *dsh* both induce ectopic dorsal furrows, *lines* has a different phenotype. (D) Mad^{B1} clones. (E) shn^{56M} clones. *shn* has the same effect as *Mad*, blocking furrow initiation in posterior margin cells. (F) $scrib^{A128}$ clones. Mutant cells overproliferate and show reduced and disorganized differentiation.

the ectopic lateral furrows associated with loss of Wg signaling (Ma and Moses 1995; Treisman and Rubin 1995), but instead produced small clones that induced overgrowth of the surrounding tissue (Figure 2, A–C). In addition, the phenotype of *schnurri* clones in the eye was indistinguishable from *Mad* and *Medea* (Wiersdorff *et al.* 1996; Das *et al.* 1998), suggesting that Dpp signaling in eye development is mediated by repression of *brinker* (Marty *et al.* 2000; Figure 2, D and E).

Three components of the Notch pathway, *fringe, kuzbanian*, and *nicastrin*, were found in our screen, but did not cause very strong adult phenotypes, perhaps explaining why other components were missed. We isolated alleles of the three eye specification genes present on the chromosome arms we screened, *eyes absent*, *dachshund*, and *sine oculis*. In addition, we found mutations in the proneural gene *atonal* (Jarman *et al.* 1994) and in the *glass* gene, which is required for normal photoreceptor differentiation and survival (Moses and Rubin 1991).

We identified several additional complementation groups as genes encoding components of the cytoskeleton. *act up/ capulet* encodes an inhibitor of actin filament polymerization that appears to retard Hh protein transport, perhaps by promoting apical constriction in the morphogenetic furrow (Benlali *et al.* 2000). *Myosin binding subunit* (*Mbs*) encodes the myosin binding subunit of myosin light chain phosphatase (Mizuno *et al.* 2002; Tan *et al.* 2003), which appears to be required to maintain photoreceptors within the eye disc epithelium (A. Lee and J. E. Treisman, unpublished results). *scribbled*, which encodes a component of septate junctions (Bilder and Perrimon 2000), is required to restrict proliferation and maintain normal photoreceptor differentiation (Figure 2F). As we have not systematically tested for failure-to-complement genes encoding cytoskeletal proteins, it is possible that more members of this class are present among our unidentified complementation groups.

***trithorax* and *Polycomb* group genes are required for normal photoreceptor differentiation:** The largest unanticipated class of genes found in our screen was a set of general transcriptional regulators of the *trithorax* group (*skd*, *kto*, *brm*, and *trx*) and the *Polycomb* group [*E(z)* and *Pc*]. The identification of *brm*, which encodes the SWI2/SNF2-related ATPase subunit of the Brm chromatin-remodeling complex, is consistent with our previous observation that photoreceptor differentiation requires Osa, another subunit of this complex (Treisman *et al.* 1997; Collins *et al.* 1999). However, the phenotype of *brm* mutations is distinct from that of *osa*. In osa^{308} mutant clones, a reduced number of photoreceptors was still able to differentiate (Figure 3, B and E), while no Elav-expressing cells were present in brm^{T485} mutant clones (Figure 3A). Most *brm* mutant clones were extremely small, but some of the alleles we isolated (brm^{T485} and brm^{T808}) allowed some growth of the mutant cells (Figure 3A). When brm^{T362} clones were generated in a *Minute* background to give the mutant cells a growth advantage, the resulting discs were very small and contained photoreceptors only within the remaining wild-type tissue (Figure 3D). Clones mutant for $moira^{1}$, which

encodes another essential Brm complex subunit, had a very similar effect (data not shown). The milder phenotype of *osa* mutant clones is consistent with our finding that Osa is present in only a subset of Brm complexes (Collins *et al.* 1999).

skd (previously named *blind spot*; Gutierrez *et al.* 2003) and *kto* encode subunits of the Drosophila mediator complex, homologs of TRAP240 and TRAP230, respectively (Treisman 2001; Janody *et al.* 2003). Their mutant phenotypes in the eye disc, while identical to each other, were distinct from those of *brm*, *mor*, and *osa*. *skd* and *kto* mutant clones showed normal growth, but had a stronger effect than *osa* on photoreceptor differentiation; only a few Elav-positive cells appeared at the posterior of the mutant clones (Figure 3C; Treisman 2001). Multiple alleles, including molecular nulls, were analyzed and shown to have the same phenotype. In a *Minute* background, loss of *skd* or *kto* reduced the size of the eye disc, but this may have been an indirect effect due to the greatly reduced numbers of photoreceptors and therefore the reduced expression of the mitogen Hh (Figure 3F).

Mutations in *trx*, homologs of which encode histone methyltransferases specific for H3 lysine 4 (Roguev *et al.* 2001; Milne *et al.* 2002; Nagy *et al.* 2002; Nakamura *et al.* 2002), showed a third distinct phenotype, affecting predominantly marginal regions of the eye disc. The allele found in our screen, trx^{67c}, behaved similarly to the amorphic allele trx^{E2}, which we used for further analysis. *trx* mutant clones in the central region of the eye disc caused only a delay in photoreceptor differentiation, but clones at the posterior or lateral margins of the disc showed a strong loss of photoreceptor differentiation accompanied by overgrowth (Figure 3G). Interestingly, clones generated in a *Minute* background and occupying almost the entire eye disc showed normal differentiation in a central posterior region, which was surrounded by regions lacking photoreceptors and expressing *dpp-lacZ* (Figure 3J). Misexpression of *dpp* was also observed in smaller *trx* clones close to the morphogenetic furrow or at the posterior margin (data not shown).

We also identified mutations in two members of the *Polycomb* group of genes, *Pc* itself and *E(z)*. The Pc and E(z) proteins are components of separate complexes required for the repression of homeotic genes. The E(z) complex has histone methyltransferase activity for lysines 9 and 27 of H3 (Cao *et al.* 2002; Czermin *et al.* 2002; Kuzmichev *et al.* 2002; Muller *et al.* 2002). Pc is a chromodomain protein that binds to methylated H3

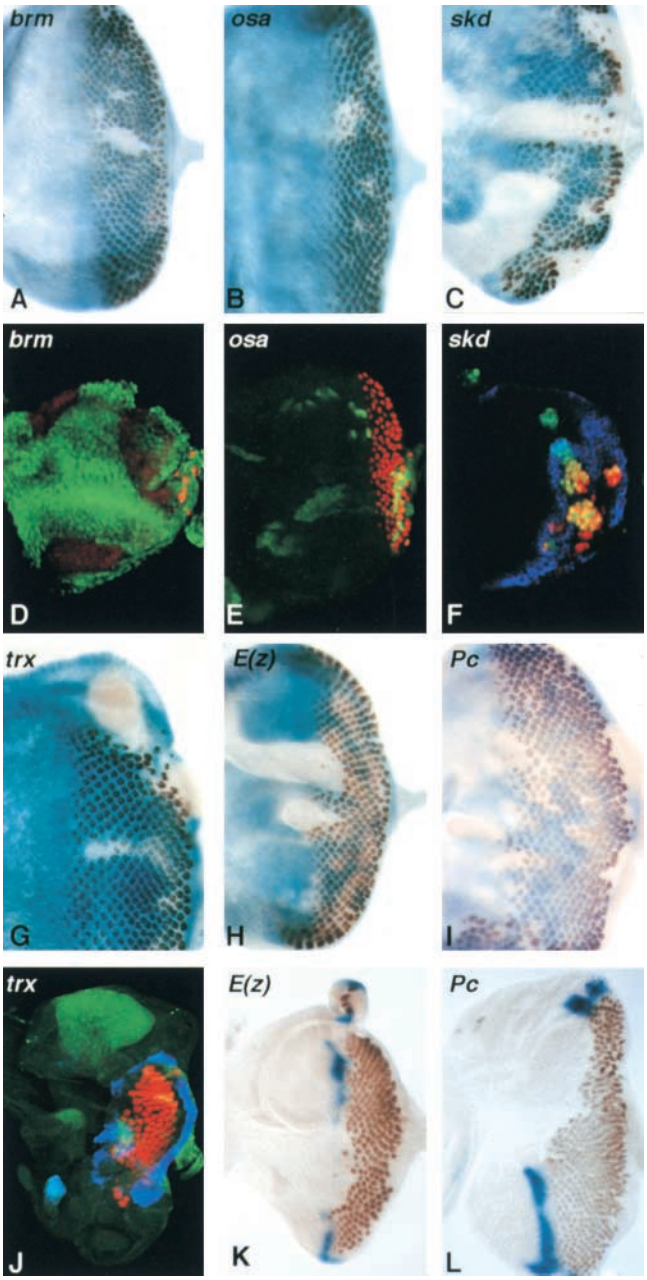

Figure 3.—*trithorax* and *Polycomb* group mutations have different effects on eye development. (A–C and G–I) Mutant clones. Wild-type tissue is marked by blue X-gal staining revealing *arm-lacZ* expression and photoreceptors are stained with anti-Elav in brown. (A) brm^{T485}. (B) osa^{308}. (C) skd^{T773}. (G) trx^{E2}. (H) $E(z)^{T643}$. (I) Pc^{T181}. *brm* affects growth strongly, *osa* and *trx* affect growth more weakly, and *skd* does not affect growth. *brm* is required for photoreceptor differentiation and *skd* strongly affects differentiation, while *osa* and *trx* have weaker effects. *E(z)* and *Pc* affect differentiation only at the morphogenetic furrow and the posterior margin. (D–F and J–L) Clones generated in a *Minute* background. (D) brm^{T362}. (E) osa^{308}. Anti-Elav is shown in red and green fluorescent protein (GFP) in green marks wild-type tissue. (F) skd^{T413}. (J) trx^{E2}. Anti-Elav is shown in red, anti-β-galactosidase reveals *dpp-lacZ* expression in blue, and GFP marks wild-type tissue. (K) $E(z)^{T643}$. (L) Pc^{T181}. Elav is stained in brown and blue X-gal staining shows *dpp-lacZ* expression. *brm* affects growth more strongly than the other mutations. *skd* shows reduced growth that is probably due to the lack of nonautonomous growth factors, as it is not seen in smaller clones. The effects of *trx*, *Pc*, and *E(z)* are restricted to particular regions of the disc.

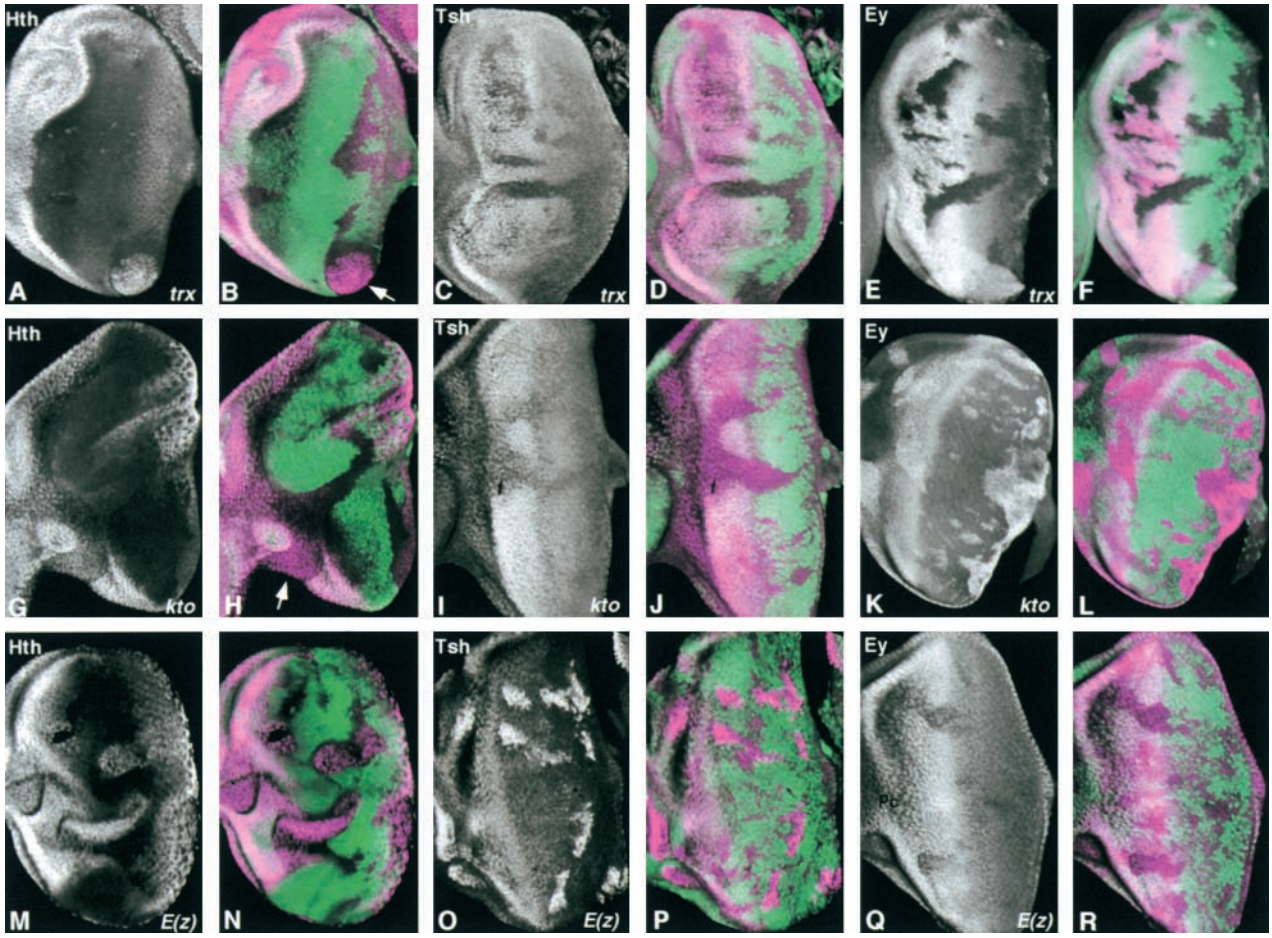

Figure 4.—Hth, Tsh, and Ey show different patterns of regulation by *trithorax* and *Polycomb* group genes. (A–F) trx^{E2} mutant clones. (G and H) kto^{T241} mutant clones. (I–L) kto^{T555} mutant clones. (M–P) $E(z)^{E4G.2}$ mutant clones. (Q and R) $E(z)^{T643}$ mutant clones. Wild-type tissue is marked with GFP in B, D, F, H, J, L, N, P, and R. Hth is white in A, G, and M and magenta in B, H, and N. Ectopic Hth is indicated by an arrow in B, and loss of Hth is indicated by an arrow in H. Tsh is white in C, I, and O and magenta in D, J, and P. Ey is white in E, K, and Q and magenta in F, L, and R. Hth is repressed by *trx* and *E(z)* but activated by *kto*. Tsh is repressed by *E(z)* but activated by *trx* and *kto*. Ey is repressed by *kto* but activated by *trx* and *E(z)*.

K27 (Cao *et al.* 2002; Fischle *et al.* 2003; Min *et al.* 2003). Loss of either gene in the eye disc showed a very similar phenotype: photoreceptor development was largely unaffected, but differentiation failed in some mutant clones centered on the morphogenetic furrow or at the posterior margin (Figure 3, H and I). Generating very large clones lacking *Pc* or *E(z)* function in a *Minute* background likewise produced loss of photoreceptor differentiation and *dpp* expression only in a region of the disc close to the furrow (Figure 3, K and L). Both complexes are thus likely to act on the same genes during eye development.

Transitions in gene expression are differently regulated by *trithorax* and *Polycomb* group genes: Eye development requires a rapid series of transitions in gene expression as the morphogenetic furrow traverses the eye disc. The *trithorax* and *Polycomb* group genes are thought to be involved in the maintenance, respectively, of activated and repressed states of gene expression. To explain their effects on photoreceptor differentiation, we examined how transitions between different gene expression domains were affected in the absence of these genes. The most anterior domain of the disc gives rise to head cuticle and expresses the homeobox gene *hth*, while the adjacent domain of the eye disc proper expresses the transcription factors encoded by *ey* and *tsh*, in addition to *hth* (Bessa *et al.* 2002). We did not observe significant changes in the expression of any of these genes in brm^{T362} or osa^{308} mutants (data not shown). In anterior trx^{E2} mutant clones, expression of *ey* and *tsh* was lost, while in clones at the posterior or lateral margins, *hth* was misexpressed (Figure 4, A–F). *skd* or *kto* mutant clones caused a reduction in *hth* and *tsh* expression, as well as autonomous maintenance of *ey* expression in all clones posterior to its normal domain (Figure 4, G–L; Treisman 2001). $E(z)^{T643}$, $E(z)^{E4G.2}$, Pc^{T181}, and Pc^{T351} clones showed a variable reduction in *ey* expression, misexpression of *hth* in some posterior clones, and strong posterior misexpression of *tsh* (Figure 4, M–R). *tsh* expression was often associated with overgrowth at

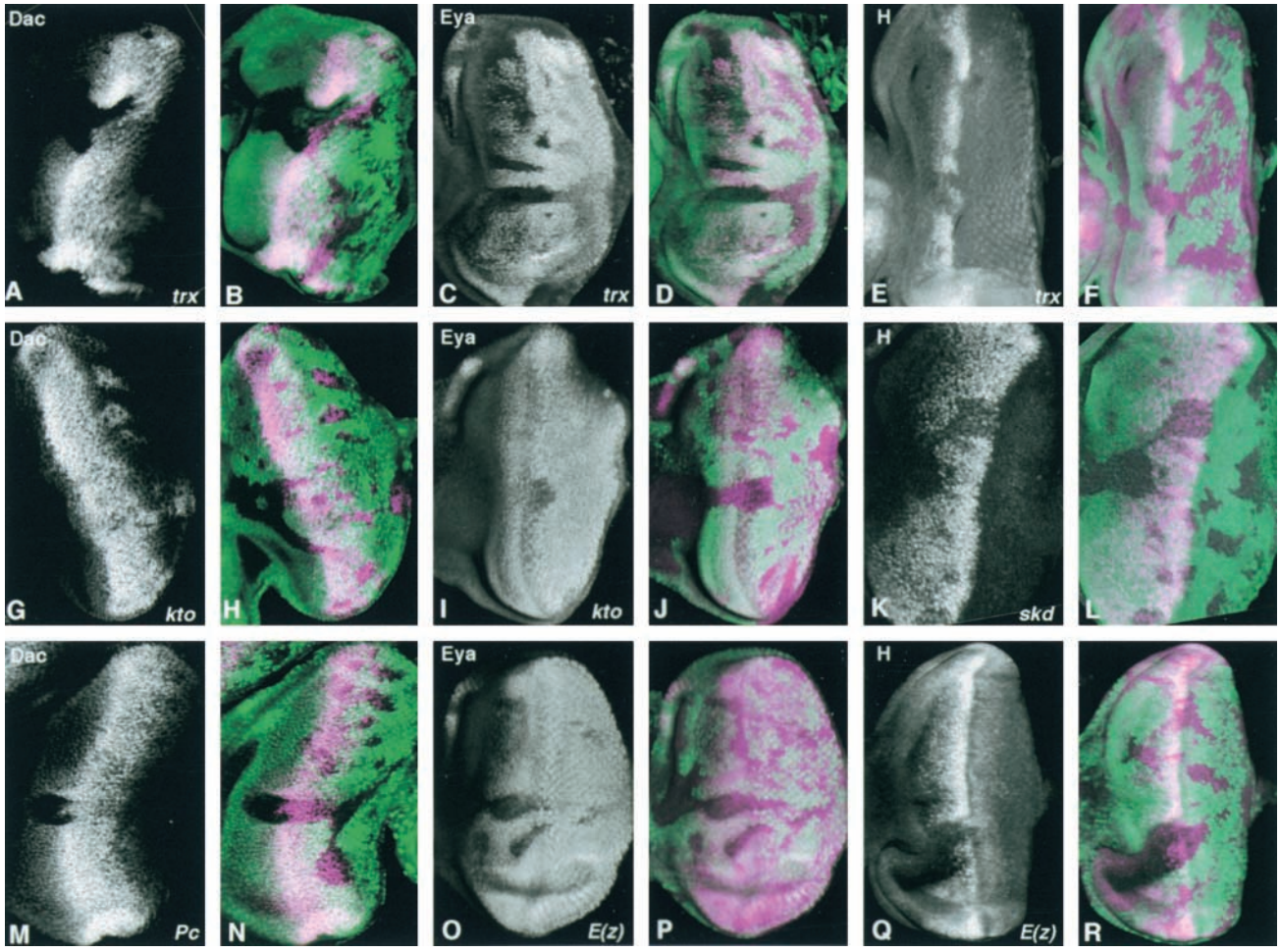

FIGURE 5.—Establishment of the preproneural domain requires both *trithorax* and *Polycomb* group genes. (A–F) trx^{E2} mutant clones. (G–J) kto^{T241} mutant clones. (K and L) skd^{T342} mutant clones. (M and N) Pc^{T181} mutant clones. (O–R) $E(z)^{T643}$ mutant clones. Wild-type tissue is marked with GFP in B, D, F, H, J, L, N, P, and R. Dac is white in A, G, and M and magenta in B, H, and N. Eya is white in C, I, and O and magenta in D, J, and P. Hairy is white in E, K, and Q and magenta in F, L, and R. High-level expression at the anterior edge of the expression domain of Eya, Dac, and H requires *trx*, *skd*, *kto*, *Pc*, and *E(z)*.

the posterior margin, and mutant cells tended to sort out to the basal region of the disc in internal clones (data not shown). This shows that the three genes are under independent regulatory control. In addition, *trithorax* and *Polycomb* group genes do not always have opposing functions: *ey* and *hth* are regulated similarly by *trx* and *E(z)/Pc* and oppositely by *trx* and *kto/skd*.

The morphogenetic furrow is preceded by a region known as the preproneural domain, in which *eya*, *so*, *dac*, and *h* are expressed (GREENWOOD and STRUHL 1999; BESSA *et al.* 2002). The initiation of this domain appeared to be delayed in *trx* mutant cells; *h* expression was reduced, and *dac* and *eya* were lost from mutant clones in the anterior region of their expression domains (Figure 5, A–F). *skd* and *kto* were also required for normal levels of *h* and *eya* expression, but in their absence *dac* was initiated normally and inappropriately maintained in posterior regions of the disc (Figure 5, G–L; TREISMAN 2001). Interestingly, *Pc* and *E(z)* mutant clones resembled *trx* mutant clones in this domain; *h* was reduced, while *eya* and *dac* were lost from anterior regions of their expression domains (Figure 5, M–R). Loss of *osa* had no apparent effect on genes expressed in this domain, but we observed reduced and posteriorly shifted *dac* expression when brm^{T362} clones were generated in a *Minute* background (data not shown).

***Ultrabithorax* is not the only target of *E(z)* and *Pc* in the eye disc:** Many of the effects of *Polycomb* group mutations have been attributed to the derepression of homeotic genes, and misexpression of homeotic genes in the eye disc can prevent eye development by inhibiting Ey function (PLAZA *et al.* 2001; BENASSAYAG *et al.* 2003). We found that both *Pc* and *E(z)* mutant clones in the eye disc strongly misexpressed the homeotic gene *Ultrabithorax* (*Ubx*), but not *Abdominal-B* (*Abd-B*) or *Antennapedia* (*Antp*; Figure 6, A, C, and H, and data not shown). However, the ectopic *Ubx* did not appear to be the cause of *hth* misexpression, as Ubx was excluded

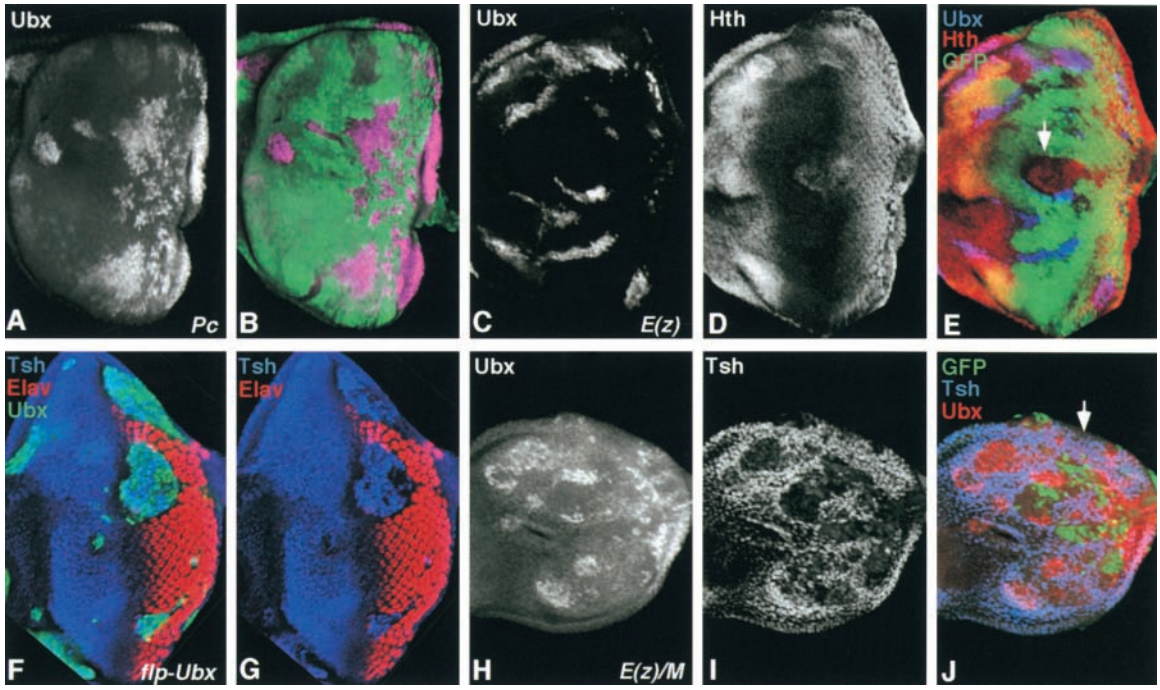

FIGURE 6.—*Ubx* is not the only target of *Polycomb* group genes in the eye disc. (A and B) Pc^{T181} mutant clones. Ubx is white in A and magenta in B, and wild-type tissue is marked with GFP in B. (C–E) $E(z)^{T643}$ mutant clones. Ubx is white in C and blue in E, Hth is white in D and red in E, and wild-type tissue is marked with GFP in E. The arrow in E indicates mutant cells that express Hth but not Ubx. (F and G) Clones expressing UAS-*Ubx* under the control of *tubulin*-GAL4. Elav is stained red, Tsh is stained blue, and Ubx is stained green in F. (H–J) $E(z)^{E4G.2}$ clones made in a *Minute* background. Ubx is white in H and red in J, Tsh is white in I and blue in J, and wild-type tissue is marked with GFP in J. The arrow in J indicates mutant cells that express Tsh but not Ubx. Ectopic Ubx can activate Tsh expression, but within *E(z)* or *Pc* mutant tissue Tsh and Hth can be misexpressed in cells that do not express Ubx.

from regions of the clones that expressed Hth (Figure 6, C–E). To test whether *Ubx* misexpression could be responsible for other aspects of the *Pc* and *E(z)* phenotypes, we generated clones ectopically expressing UAS-*Ubx* from a *tubulin*-GAL4 driver. Such clones were able to induce *tsh* expression and block photoreceptor differentiation in posterior regions of the eye disc (Figure 6, F and G). However, in discs largely lacking *Pc* or *E(z)* function, Tsh was misexpressed in some cells that did not express Ubx (Figure 6, H–J). We conclude that Pc and E(z) are likely to have targets in addition to *Ubx* in the eye disc and might themselves directly regulate *hth* and/or *tsh*. Misexpression of Hth and Tsh could be responsible for the downregulation of *h*, *eya*, and *dac* in *Pc* and *E(z)* mutant clones (BESSA *et al.* 2002).

DISCUSSION

Genes required for photoreceptor differentiation: Using a systematic genetic screen, we have attempted to define the set of genes required for photoreceptors to differentiate in their normal numbers. The known genes that we identified include components of all the pathways previously shown to be involved in photoreceptor differentiation, as well as genes encoding the transcription factors Eya, So, Dac, Ato, and Glass. We have shown that two additional genes, *sightless* and *hyperplastic discs*, encode novel components of the Hh pathway (LEE and TREISMAN 2001; LEE *et al.* 2002). It is possible that other components of these pathways will be found among the complementation groups that are still unidentified. We have also found evidence that differentiation can be affected by defects in the cytoskeleton that may alter cell shape or cell motility (BENLALI *et al.* 2000; A. LEE and J. E. TREISMAN, unpublished results). In addition, several members of the *trithorax* and *Polycomb* groups of transcriptional regulators had striking effects on photoreceptor differentiation and were identified in our screen.

The screen was probably not fully saturating. Although we were able to find multiple alleles even of quite small genes such as *act up* [424 amino acids (aa)], *so* (416 aa), *fringe* (412 aa), *downstream of receptor kinases* (211 aa), and *ras1* (189 aa), many of the genes we found were identified only by single alleles. In addition to size, the probability of our finding mutations in a gene depended on the severity of its phenotype and was reduced if only clones in a particular region of the eye disc had a visible phenotype; for instance, *dac* clones cause photoreceptor loss only if they occur at the poste-

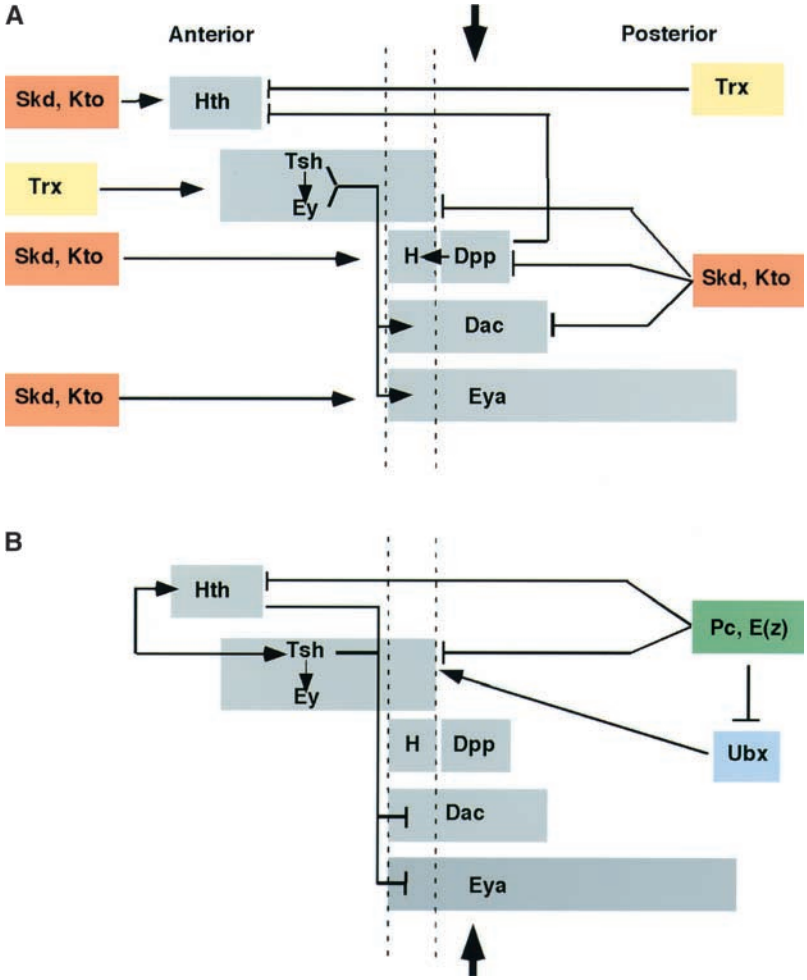

FIGURE 7.—Effects of *trithorax* and *Polycomb* group genes on domains of transcription factor expression in the eye disc. Anterior-posterior domains of gene expression are depicted as boxes, with anterior to the left. Dashed lines delimit the preproneural domain and thick arrows mark the position of the morphogenetic furrow. (A) The effects of *trithorax* group genes. Trx activates *ey* and *tsh* and may therefore indirectly activate genes in the preproneural domain, since Ey and Tsh in combination can activate *dac* and *eya* (BESSA *et al.* 2002). Skd and Kto activate *hth* in the anterior of the disc and repress *ey*, *dac*, and *dpp* in posterior regions. They also contribute to the activation of *h* and *eya*, perhaps by promoting the effects of Dpp on transcription. (B) The effects of *Polycomb* group genes. Pc and E(z) repress *hth* and *tsh* posteriorly to their normal expression domains, as well as repress inappropriate expression of *Ubx*; Ubx may contribute to the activation of *tsh*. Ectopic Hth and Tsh may be responsible for the repression of *eya* and *dac* (BESSA *et al.* 2002). Arrows in this diagram do not imply that any of the interactions are direct.

rior margin. In addition, we could not find redundant genes, such as *rhomboid* and *roughoid* (WASSERMAN *et al.* 2000). Finally, we were unable to screen the regions proximal to the FRT sites or the fourth chromosome and have not yet screened the X chromosome. However, it may be possible to draw some conclusions about the prevalence of certain classes of genes. Among our unidentified mutations, none have a phenotype resembling that of the eye specification genes *eya*, *so*, and *dac*. Unless additional genes of this class are clustered in a region that was not screened, they are unlikely to be very numerous. It has been proposed that *optix* also encodes an eye specification gene (SEIMIYA and GEHRING 2000). However, although we found five alleles of *so*, which encodes a smaller protein than *optix* does, we did not find any gene with a similar phenotype mapping to the same region as *optix* (44A).

***Polycomb* group genes repress *Ubx*, *tsh*, and *hth*:** We observed very similar phenotypes in clones mutant for *Pc* or *E(z)*, which encode components of two distinct complexes implicated in transcriptional repression. Although we used likely null alleles for both genes (see MATERIALS AND METHODS), the phenotype of *E(z)* clones appeared slightly stronger, with a greater likelihood of derepressing *hth* in posterior regions of the eye disc. The E(z) protein has been shown to act as a histone methyltransferase for H3 K27 within a complex that also includes Extra sex combs (Esc), Suppressor of zeste 12 [Su(z)12], and the histone-binding protein NURF-55 (CAO *et al.* 2002; CZERMIN *et al.* 2002; KUZMICHEV *et al.* 2002; MULLER *et al.* 2002). *esc* appears to act only early in embryonic development (STRUHL and BROWER 1982; SATHE and HARTE 1995; SIMON *et al.* 1995), while *E(z)* and *Su(z)12* are continuously required to repress inappropriate homeotic gene expression in wing imaginal discs (JONES and GELBART 1990; LAJEUNESSE and SHEARN 1996; BIRVE *et al.* 2001; CZERMIN *et al.* 2002; MULLER *et al.* 2002). The core PRC1 complex contains Pc, as well as Ph, Psc, and dRing1, and can prevent SWI/SNF complexes from binding to a chromatin template (FRANCIS *et al.* 2001). *Pc*, *Psc*, and *ph* are all required to prevent homeotic gene misexpression in wing discs; however, *Psc* and *ph* act redundantly with closely related adjacent genes (BEUCHLE *et al.* 2001). The two complexes are thought to be linked through binding of the Pc chromodomain to K27-methylated H3 (CAO *et al.* 2002; FISCHLE *et al.* 2003; MIN *et al.* 2003). The stronger phenotype of *E(z)* mutations in the eye disc might suggest that

methylation of H3 K27 can recruit other proteins in addition to Pc.

In the eye disc, loss of *E(z)* or *Pc* leads to misexpression of the homeotic gene *Ubx*, but this does not seem to account for the entire phenotype. Although Ubx is sufficient to turn on *tsh* ectopically, misexpression of *hth* and *tsh* can occur in *E(z)* or *Pc* clones in which *Ubx* is not misexpressed. This suggests that *hth* and *tsh* are either direct targets of Pc/E(z)-mediated repression or targets of a downstream gene other than *Ubx*, possibly one of the homeotic genes that we did not examine (Figure 7B). Tsh misexpression would be sufficient to explain the suppression of photoreceptor differentiation in clones close to the morphogenetic furrow, since it is able to maintain expression of Hth and Ey and, in combination with them, to repress *eya* (Bessa *et al.* 2002). Misexpression of Tsh can also account for overgrowth of *Pc* or *E(z)* mutant cells at the posterior margin of the eye disc (Bessa *et al.* 2002).

***trithorax* group genes have a variety of distinct functions:** *trithorax* group genes were initially identified as suppressors of *Polycomb* phenotypes (Kennison and Tamkun 1988) and are therefore thought to contribute to the activation of homeotic gene expression. Some members of the group encode components of the Brahma chromatin-remodeling complex (Tamkun *et al.* 1992; Papoulas *et al.* 1998; Collins *et al.* 1999; Crosby *et al.* 1999), others encode components of the mediator coactivation complex (Boube *et al.* 2000; Treisman 2001), and still others encode histone methyltransferases (Roguev *et al.* 2001; Beisel *et al.* 2002; Czermin *et al.* 2002; Milne *et al.* 2002; Nagy *et al.* 2002; Nakamura *et al.* 2002) or less-well-characterized proteins (Farkas *et al.* 1994; Adamson and Shearn 1996; Daubresse *et al.* 1999; Gildea *et al.* 2000; Therrien *et al.* 2000; Calgaro *et al.* 2002; Gutierrez *et al.* 2003). Our analysis shows that in addition to their distinct biochemical functions, members of the *trithorax* group act on different sets of target genes during eye development and can also have different effects on the same target genes. Components of the Brahma complex are strongly required for cell growth and/or survival; *brm* and *mor*, but not *osa*, are also absolutely required for photoreceptor differentiation. However, these three genes do not seem to be required for the restricted expression in anterior-posterior domains of the eye disc of the transcription factors we examined. In contrast, the mediator complex subunits encoded by *skd* and *kto* are not required for cell proliferation, although they are strongly required for photoreceptor differentiation (Treisman 2001). *trx*, which encodes a histone methyltransferase (Czermin *et al.* 2002), is required primarily for the normal development of marginal regions of the disc. We have not seen any significant effect on photoreceptor differentiation in clones mutant for *kismet*^{1}, which encodes chromodomain proteins (Daubresse *et al.* 1999; Therrien *et al.* 2000), or *ash2*^{1}, which encodes a PHD protein (Adamson and Shearn 1996; data not shown). These differences are unlikely to be due to different expression patterns of the trithorax group genes, as Trx, Skd, Kto, and Osa are ubiquitously expressed in the eye disc (Kuzin *et al.* 1994; Treisman *et al.* 1997; Janody *et al.* 2003).

The effects of these genes on the rapid transitions between domains of expression of different transcription factors are of particular interest (Figure 7A). In the most anterior region of the eye disc, *hth* expression is enhanced by *skd* and *kto*. The domain just posterior to this also expresses *tsh* and *ey*, and activation of both of these genes requires *trx*. However, *skd* and *kto* have opposite effects on the two genes, enhancing *tsh* expression and preventing the maintenance of *ey* expression in posterior cells. Since Hth and Tsh can positively regulate each other's expression (Bessa *et al.* 2002), it is possible that only one of these genes is directly dependent on *skd* and *kto*. Next, *dac* and *h* are activated transiently and *eya* is activated and sustained. The establishment of both *dac* and *eya* is delayed in *trx* mutant clones, and *h* expression is reduced. This delay in establishing the preproneural domain may be due to the failure to activate *ey* and *tsh* earlier in development, since Ey and Tsh combine to activate *eya* (Bessa *et al.* 2002). The effect of *Pc* or *E(z)* mutations on *eya*, *dac*, and *h* appears very similar to the effect of *trx* mutations. However, in *Pc* or *E(z)* clones, the delay in *eya* and *dac* expression is likely to be caused by the failure to repress *tsh* and *hth*, since the combination of these two proteins has been shown to repress genes expressed in the preproneural domain (Bessa *et al.* 2002). *skd* and *kto* clones also show a reduction in *h* and anterior *eya* expression, but an inappropriate maintenance of *dac* and *dpp* (Treisman 2001). These mediator complex components may thus contribute both to the activation of genes such as *h* in the preproneural domain and to the activation of unknown genes that shut off the expression of *ey*, *dac*, and *dpp*. Alternatively, *skd* and *kto* could be directly involved in the repression of these genes. Finally, *trx* is important to prevent misexpression of *hth* in cells near the posterior and lateral margins. Although Dpp normally represses *hth* (Bessa *et al.* 2002), in *trx* mutant clones *dpp* and *hth* are both inappropriately expressed in marginal cells. This may reflect a role for *trx* in the process of morphogenetic furrow initiation, perhaps contributing to the ability of *dpp* to control gene expression.

Further study will be needed to determine which genes are direct targets of each trithorax group protein. However, our results point to a strong specificity of these general transcriptional regulators, suggesting that they may be specialized to mediate the effects of particular signaling pathways (Collins and Treisman 2000; Janody *et al.* 2003) or to control specific subsets of downstream genes.

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LITERATURE CITED

Adamson, A. L., and A. Shearn, 1996 Molecular genetic analysis of *Drosophila ash2*, a member of the *trithorax* group required for imaginal disc pattern formation. Genetics **144:** 621–633.

Baker, N. E., and S.-Y. Yu, 1997 Proneural function of neurogenic genes in the developing *Drosophila* eye. Curr. Biol. **7:** 122–132.

Baonza, A., and M. Freeman, 2001 Notch signalling and the initiation of neural development in the *Drosophila* eye. Development **128:** 3889–3898.

Beisel, C., A. Imhof, J. Greene, E. Kremmer and F. Sauer, 2002 Histone methylation by the *Drosophila* epigenetic transcriptional regulator Ash1. Nature **419:** 857–862.

Benassayag, C., S. Plaza, P. Callaerts, J. Clements, Y. Romeo *et al.*, 2003 Evidence for a direct functional antagonism of the selector genes *proboscipedia* and *eyeless* in *Drosophila* head development. Development **130:** 575–586.

Benlali, A., I. Draskovic, D. J. Hazelett and J. E. Treisman, 2000 *act up* controls actin polymerization to alter cell shape and restrict Hedgehog signaling in the *Drosophila* eye disc. Cell **101:** 271–281.

Bessa, J., B. Gebelein, F. Pichaud, F. Casares and R. S. Mann, 2002 Combinatorial control of *Drosophila* eye development by *eyeless*, *homothorax*, and *teashirt*. Genes Dev. **16:** 2415–2427.

Beuchle, D., G. Struhl and J. Muller, 2001 Polycomb group proteins and heritable silencing of *Drosophila Hox* genes. Development **128:** 993–1004.

Bilder, D., and N. Perrimon, 2000 Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. Nature **403:** 676–680.

Birve, A., A. K. Sengupta, D. Beuchle, J. Larsson, J. A. Kennison *et al.*, 2001 *Su(z)12*, a novel *Drosophila Polycomb* group gene that is conserved in vertebrates and plants. Development **128:** 3371–3379.

Blackman, R. K., M. Sanicola, L. A. Raftery, T. Gillevet and W. M. Gelbart, 1991 An extensive 3′ *cis*-regulatory region directs the imaginal disc expression of *decapentaplegic*, a member of the TGF-β family in *Drosophila*. Development **111:** 657–665.

Bonini, N. M., W. M. Leiserson and S. Benzer, 1993 The *eyes absent* gene: genetic control of cell survival and differentiation in the developing *Drosophila* eye. Cell **72:** 379–395.

Boube, M., C. Faucher, L. Joulia, D. L. Cribbs and H. M. Bourbon, 2000 *Drosophila* homologs of transcriptional mediator complex subunits are required for adult cell and segment identity specification. Genes Dev. **14:** 2906–2917.

Boube, M., L. Joulia, D. L. Cribbs and H. M. Bourbon, 2002 Evidence for a mediator of RNA polymerase II transcriptional regulation conserved from yeast to man. Cell **110:** 143–151.

Cagan, R. L., and D. F. Ready, 1989 *Notch* is required for successive cell decisions in the developing *Drosophila* retina. Genes Dev. **3:** 1099–1112.

Calgaro, S., M. Boube, D. L. Cribbs and H. M. Bourbon, 2002 The Drosophila gene *taranis* encodes a novel *trithorax* group member potentially linked to the cell cycle regulatory apparatus. Genetics **160:** 547–560.

Cao, R., L. Wang, H. Wang, L. Xia, H. Erdjument-Bromage *et al.*, 2002 Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science **298:** 1039–1043.

Carroll, S. B., A. Laughon and B. S. Thalley, 1988 Expression, function, and regulation of the hairy segmentation protein in the *Drosophila* embryo. Genes Dev. **2:** 883–890.

Castelli-Gair, J., S. Greig, G. Micklem and M. Akam, 1994 Dissecting the temporal requirements for homeotic gene function. Development **120:** 1983–1995.

Chen, R., M. Amoui, Z. Zhang and G. Mardon, 1997 Dachshund and eyes absent proteins form a complex and function synergistically to induce ectopic eye development in *Drosophila*. Cell **91:** 893–904.

Collins, R. T., and J. E. Treisman, 2000 Osa-containing Brahma chromatin remodeling complexes are required for the repression of Wingless target genes. Genes Dev. **14:** 3140–3152.

Collins, R. T., T. Furukawa, N. Tanese and J. E. Treisman, 1999 Osa associates with the Brahma chromatin remodeling complex and promotes the activation of some target genes. EMBO J. **18:** 7029–7040.

Crosby, M. A., C. Miller, T. Alon, K. L. Watson, C. P. Verrijzer *et al.*, 1999 The *trithorax* group gene *moira* encodes a brahma-associated putative chromatin-remodeling factor in *Drosophila melanogaster*. Mol. Cell. Biol. **19:** 1159–1170.

Czermin, B., R. Melfi, D. McCabe, V. Seitz, A. Imhof *et al.*, 2002 *Drosophila* enhancer of Zeste/ESC complexes have a histone H3 methyltransferase activity that marks chromosomal Polycomb sites. Cell **111:** 185–196.

Czerny, T., G. Halder, U. Kloter, A. Souabni, W. J. Gehring *et al.*, 1999 *twin of eyeless*, a second *Pax-6* gene of *Drosophila*, acts upstream of *eyeless* in the control of eye development. Mol. Cell **3:** 297–307.

Das, P., L. L. Maduzia, H. Wang, A. L. Finelli, S. H. Cho *et al.*, 1998 The *Drosophila* gene *Medea* demonstrates the requirement for different classes of Smads in dpp signaling. Development **125:** 1519–1528.

Daubresse, G., R. Deuring, L. Moore, O. Papoulas, I. Zakrajsek *et al.*, 1999 The *Drosophila kismet* gene is related to chromatin-remodeling factors and is required for both segmentation and segment identity. Development **126:** 1175–1187.

Dominguez, M., 1999 Dual role for *hedgehog* in the regulation of the proneural gene *atonal* during ommatidia development. Development **126:** 2345–2353.

Dominguez, M., M. Brunner, E. Hafen and K. Basler, 1996 Sending and receiving the hedgehog signal: control by the *Drosophila* Gli protein Cubitus interruptus. Science **272:** 1621–1625.

Farkas, G., J. Gausz, M. Galloni, G. Reuter, H. Gyurkovics *et al.*, 1994 The *Trithorax-like* gene encodes the *Drosophila* GAGA factor. Nature **371:** 806–808.

Fischle, W., Y. Wang, S. A. Jacobs, Y. Kim, C. D. Allis *et al.*, 2003 Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. Genes Dev. **17:** 1870–1881.

Francis, N. J., A. J. Saurin, Z. Shao and R. E. Kingston, 2001 Reconstitution of a functional core polycomb repressive complex. Mol. Cell **8:** 545–556.

Freeman, M., 1996 Reiterative use of the EGF receptor triggers differentiation of all cell types in the *Drosophila* eye. Cell **87:** 651–660.

Gildea, J. J., R. Lopez and A. Shearn, 2000 A screen for new *trithorax* group genes identified *little imaginal discs*, the *Drosophila melanogaster* homologue of human retinoblastoma binding protein 2. Genetics **156:** 645–663.

Gindhart, J. G., Jr., and T. C. Kaufman, 1995 Identification of *Polycomb* and *trithorax* group responsive elements in the regulatory region of the Drosophila homeotic gene *Sex combs reduced*. Genetics **139:** 797–814.

Greenwood, S., and G. Struhl, 1999 Progression of the morphogenetic furrow in the *Drosophila* eye: the roles of Hedgehog, Decapentaplegic and the Raf pathway. Development **126:** 5795–5808.

Gutierrez, L., M. Zurita, J. A. Kennison and M. Vazquez, 2003 The *Drosophila trithorax* group gene *tonalli* (*tna*) interacts genetically with the Brahma remodeling complex and encodes an SP-RING finger protein. Development **130:** 343–354.

Halder, G., P. Callaerts, S. Flister, U. Walldorf, U. Kloter *et al.*, 1998 Eyeless initiates the expression of both *sine oculis* and *eyes absent* during *Drosophila* compound eye development. Development **125:** 2181–2191.

Hatini, V., P. Bokor, R. Goto-Mandeville and S. DiNardo, 2000

Tissue- and stage-specific modulation of Wingless signaling by the segment polarity gene *lines*. Genes Dev. **14:** 1364–1376.

Hazelett, D. J., M. Bourouis, U. Walldorf and J. E. Treisman, 1998 *decapentaplegic* and *wingless* are regulated by *eyes absent* and *eyegone* and interact to direct the pattern of retinal differentiation in the eye disc. Development **125:** 3741–3751.

Heberlein, U., T. Wolff and G. M. Rubin, 1993 The TGFβ homolog *dpp* and the segment polarity gene *hedgehog* are required for propagation of a morphogenetic wave in the *Drosophila* retina. Cell **75:** 913–926.

Holstege, F. C. P., E. G. Jennings, J. J. Wyrick, T. I. Lee, C. J. Hengartner *et al.*, 1998 Dissecting the regulatory circuitry of a eukaryotic genome. Cell **95:** 717–728.

Janody, F., Z. Martirosyan, A. Benlali and J. E. Treisman, 2003 Two subunits of the *Drosophila* mediator complex act together to control cell affinity. Development **130:** 3691–3701.

Jarman, A. P., E. H. Grell, L. Ackerman, L. Y. Jan and Y. N. Jan, 1994 *atonal* is the proneural gene for *Drosophila* photoreceptors. Nature **369:** 398–400.

Jones, R. S., and W. M. Gelbart, 1990 Genetic analysis of the *enhancer of zeste* locus and its role in gene regulation in *Drosophila melanogaster*. Genetics **126:** 185–199.

Kennison, J. A., and J. W. Tamkun, 1988 Dosage-dependent modifiers of *Polycomb* and *Antennapedia* mutations in *Drosophila*. Proc. Natl. Acad. Sci. USA **85:** 8136–8140.

Kurant, E., C. Y. Pai, R. Sharf, N. Halachmi, Y. H. Sun *et al.*, 1998 *Dorsotonals/homothorax*, the *Drosophila* homologue of *meis1*, interacts with *extradenticle* in patterning of the embryonic PNS. Development **125:** 1037–1048.

Kuzin, B., S. Tillib, Y. Sedkov, L. Mizrokhi and A. Mazo, 1994 The *Drosophila trithorax* gene encodes a chromosomal protein and directly regulates the region-specific homeotic gene *fork head*. Genes Dev. **8:** 2478–2490.

Kuzmichev, A., K. Nishioka, H. Erdjument-Bromage, P. Tempst and D. Reinberg, 2002 Histone methyltransferase activity associated with a human multiprotein complex containing the Enhancer of Zeste protein. Genes Dev. **16:** 2893–2905.

LaJeunesse, D., and A. Shearn, 1996 *E(z)*: A *polycomb* group gene or a *trithorax* group gene? Development **122:** 2189–2197.

Lee, J. D., and J. E. Treisman, 2001 Sightless has homology to transmembrane acyltransferases and is required to generate active Hedgehog protein. Curr. Biol. **11:** 1147–1152.

Lee, J. D., K. Amanai, A. Shearn and J. E. Treisman, 2002 The ubiquitin ligase Hyperplastic discs negatively regulates *hedgehog* and *decapentaplegic* expression by independent mechanisms. Development **129:** 5697–5706.

Ma, C., and K. Moses, 1995 *wingless* and *patched* are negative regulators of the morphogenetic furrow and can affect tissue polarity in the developing *Drosophila* compound eye. Development **121:** 2279–2289.

Ma, C., Y. Zhou, P. A. Beachy and K. Moses, 1993 The segment polarity gene *hedgehog* is required for progression of the morphogenetic furrow in the developing *Drosophila* eye. Cell **75:** 927–938.

Mardon, G., N. M. Solomon and G. M. Rubin, 1994 *dachsund* encodes a nuclear protein required for normal eye and leg development in *Drosophila*. Development **120:** 3473–3486.

Martens, J. A., and F. Winston, 2002 Evidence that Swi/Snf directly represses transcription in *S. cerevisiae*. Genes Dev. **16:** 2231–2236.

Marty, T., B. Muller, K. Basler and M. Affolter, 2000 Schnurri mediates Dpp-dependent repression of *brinker* transcription. Nat. Cell Biol. **2:** 745–749.

Milne, T. A., S. D. Briggs, H. W. Brock, M. E. Martin, D. Gibbs *et al.*, 2002 MLL targets SET domain methyltransferase activity to Hox gene promoters. Mol. Cell **10:** 1107–1117.

Min, J., Y. Zhang and R. M. Xu, 2003 Structural basis for specific binding of Polycomb chromodomain to histone H3 methylated at Lys 27. Genes Dev. **17:** 1823–1828.

Mizuno, T., K. Tsutsui and Y. Nishida, 2002 *Drosophila* myosin phosphatase and its role in dorsal closure. Development **129:** 1215–1223.

Morata, G., and P. Ripoll, 1975 *Minutes*: mutants autonomously affecting cell division rate in *Drosophila*. Dev. Biol. **42:** 211–221.

Moses, K., and G. M. Rubin, 1991 *glass* encodes a site-specific DNA-binding protein that is regulated in response to positional signals in the developing *Drosophila* eye. Genes Dev. **5:** 583–593.

Muller, J., C. M. Hart, N. J. Francis, M. L. Vargas, A. Sengupta *et al.*, 2002 Histone methyltransferase activity of a *Drosophila* Polycomb group repressor complex. Cell **111:** 197–208.

Nagy, P. L., J. Griesenbeck, R. D. Kornberg and M. L. Cleary, 2002 A trithorax-group complex purified from Saccharomyces cerevisiae is required for methylation of histone H3. Proc. Natl. Acad. Sci. USA **99:** 90–94.

Nakamura, T., T. Mori, S. Tada, W. Krajewski, T. Rozovskaia *et al.*, 2002 ALL-1 is a histone methyltransferase that assembles a supercomplex of proteins involved in transcriptional regulation. Mol. Cell **10:** 1119–1128.

Narlikar, G. J., H. Y. Fan and R. E. Kingston, 2002 Cooperation between complexes that regulate chromatin structure and transcription. Cell **108:** 475–487.

Newsome, T. P., B. Asling and B. J. Dickson, 2000 Analysis of *Drosophila* photoreceptor axon guidance in eye-specific mosaics. Development **127:** 851–860.

Ng, H. H., F. Robert, R. A. Young and K. Struhl, 2003 Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. Mol. Cell **11:** 709–719.

Orlando, V., 2003 Polycomb, epigenomes, and control of cell identity. Cell **112:** 599–606.

Papoulas, O., S. J. Beek, S. L. Moseley, C. M. McCallum, M. Sarte *et al.*, 1998 The *Drosophila* trithorax group proteins BRM, ASH1 and ASH2 are subunits of distinct protein complexes. Development **125:** 3955–3966.

Pignoni, F., B. Hu, K. H. Zavitz, J. Xiao, P. A. Garrity *et al.*, 1997 The eye specification proteins so and eya form a complex and regulate multiple steps in *Drosophila* eye development. Cell **91:** 881–892.

Plaza, S., F. Prince, J. Jaeger, U. Kloter, S. Flister *et al.*, 2001 Molecular basis for the inhibition of *Drosophila* eye development by Antennapedia. EMBO J. **20:** 802–811.

Quiring, R., U. Walldorf, U. Kloter and W. J. Gehring, 1994 Homology of the *eyeless* gene of *Drosophila* to the *Small eye* gene in mice and *Aniridia* in humans. Science **265:** 785–789.

Rachez, C., and L. P. Freedman, 2001 Mediator complexes and transcription. Curr. Opin. Cell Biol. **13:** 274–280.

Ready, D. F., T. E. Hanson and S. Benzer, 1976 Development of the *Drosophila* retina, a neurocrystalline lattice. Dev. Biol. **53:** 217–240.

Robinow, S., and K. White, 1991 Characterization and spatial distribution of the ELAV protein during *Drosophila melanogaster* development. J. Neurobiol. **22:** 443–461.

Roguev, A., D. Schaft, A. Shevchenko, W. W. Pijnappel, M. Wilm *et al.*, 2001 The Saccharomyces cerevisiae Set1 complex includes an Ash2 homologue and methylates histone 3 lysine 4. EMBO J. **20:** 7137–7148.

Royet, J., and R. Finkelstein, 1997 Establishing primordia in the *Drosophila* eye-antennal imaginal disc: the roles of *decapentaplegic*, *wingless* and *hedgehog*. Development **124:** 4793–4800.

Santos-Rosa, H., R. Schneider, A. J. Bannister, J. Sherriff, B. E. Bernstein *et al.*, 2002 Active genes are tri-methylated at K4 of histone H3. Nature **419:** 407–411.

Sathe, S. S., and P. J. Harte, 1995 The *Drosophila* extra sex combs protein contains WD motifs essential for its function as a repressor of homeotic genes. Mech. Dev. **52:** 77–87.

Schwartz, C., J. Locke, C. Nishida and T. B. Kornberg, 1995 Analysis of *cubitus interruptus* regulation in *Drosophila* embryos and imaginal disks. Development **121:** 1625–1635.

Seimiya, M., and W. J. Gehring, 2000 The *Drosophila* homeobox gene *optix* is capable of inducing ectopic eyes by an *eyeless*-independent mechanism. Development **127:** 1879–1886.

Simon, J., 1995 Locking in stable states of gene expression: transcriptional control during *Drosophila* development. Curr. Opin. Cell Biol. **7:** 376–385.

Simon, J., D. Bornemann, K. Lunde and C. Schwartz, 1995 The extra sex combs product contains WD40 repeats and its time of action implies a role distinct from other Polycomb group products. Mech. Dev. **53:** 197–208.

Struhl, G., and D. Brower, 1982 Early role of the *esc*+ gene product in the determination of segments in *Drosophila*. Cell **31:** 285–292.

Strutt, D. I., and M. Mlodzik, 1996 The regulation of *hedgehog* and *decapentaplegic* during *Drosophila* eye imaginal disc development. Mech. Dev. **58:** 39–50.

Tamkun, J. W., R. Deuring, M. P. Scott, M. Kissinger, A. M. Pattatucci *et al.*, 1992 *brahma*: a regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell **68:** 561–572.

Tan, C., B. Stronach and N. Perrimon, 2003 Roles of myosin phosphatase during *Drosophila* development. Development **130:** 671–681.

Therrien, M., D. K. Morrison, A. M. Wong and G. M. Rubin, 2000 A genetic screen for modifiers of a kinase suppressor of Ras-dependent rough eye phenotype in Drosophila. Genetics **156:** 1231–1242.

Tio, M., and K. Moses, 1997 The *Drosophila* TGFalpha homolog Spitz acts in photoreceptor recruitment in the developing retina. Development **124:** 343–351.

Treisman, J. E., 2001 *Drosophila* homologues of the transcriptional coactivation complex subunits TRAP240 and TRAP230 are required for identical processes in eye-antennal disc development. Development **128:** 603–615.

Treisman, J. E., and G. M. Rubin, 1995 *wingless* inhibits morphogenetic furrow movement in the *Drosophila* eye disc. Development **121:** 3519–3527.

Treisman, J. E., A. Luk, G. M. Rubin and U. Heberlein, 1997 *eyelid* antagonizes *wingless* signaling during *Drosophila* development and has homology to the Bright family of DNA-binding proteins. Genes Dev. **11:** 1949–1962.

Vincent, J. P., C. H. Girdham and P. H. O'Farrell, 1994 A cell-autonomous, ubiquitous marker for the analysis of *Drosophila* genetic mosaics. Dev. Biol. **164:** 328–331.

Wang, H., R. Cao, L. Xia, H. Erdjument-Bromage, C. Borchers *et al.*, 2001 Purification and functional characterization of a histone H3-lysine 4-specific methyltransferase. Mol. Cell **8:** 1207–1217.

Wasserman, J. D., S. Urban and M. Freeman, 2000 A family of *rhomboid*-like genes: *Drosophila rhomboid-1* and *roughoid/rhomboid-3* cooperate to activate EGF receptor signaling. Genes Dev. **14:** 1651–1663.

Wehrli, M., S. T. Dougan, K. Caldwell, L. O'Keefe, S. Schwartz *et al.*, 2000 *arrow* encodes an LDL-receptor-related protein essential for Wingless signalling. Nature **407:** 527–530.

White, R. A., and M. Wilcox, 1984 Protein products of the *bithorax* complex in *Drosophila*. Cell **39:** 163–171.

Wiersdorff, V., T. Lecuit, S. M. Cohen and M. Mlodzik, 1996 *Mad* acts downstream of dpp receptors, revealing a differential requirement for *dpp* signaling in initiation and propagation of morphogenesis in the *Drosophila* eye. Development **122:** 2153–2162.

Wu, J., and S. M. Cohen, 2000 Proximal distal axis formation in the *Drosophila* leg: distinct functions of Teashirt and Homothorax in the proximal leg. Mech. Dev. **94:** 47–56.

Xu, T., and G. M. Rubin, 1993 Analysis of genetic mosaics in developing and adult *Drosophila* tissues. Development **117:** 1223–1237.

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