RESEARCH COMMUNICATION

The *Drosophila* microRNA *iab-4* causes a dominant homeotic transformation of halteres to wings

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The *Drosophila* Bithorax Complex encodes three well-characterized homeodomain proteins that direct segment identity, as well as several noncoding RNAs of unknown function. Here, we analyze the *iab-4* locus, which produces the microRNAs *iab-4-5p* and *iab-4-3p*. *iab-4* is analogous to *miR-196* in vertebrate Hox clusters. Previous studies demonstrate that *miR-196* interacts with the *Hoxb8* 3' untranslated region. Evidence is presented that *miR-iab-4-5p* directly inhibits *Ubx* activity in vivo. Ectopic expression of *mir-iab-4-5p* attenuates endogenous *Ubx* protein accumulation and induces a classical homeotic mutant phenotype: the transformation of halteres into wings. These findings provide the first evidence for a noncoding homeotic gene and raise the possibility that other such genes occur within the Bithorax complex. We also discuss the regulation of *mir-iab-4* expression during development.

Received September 6, 2005; revised version accepted October 31, 2005.

Classical genetic studies suggest that the Bithorax Complex (BX-C) contains as many as nine homeotic genes (Lewis 1978). However, only three encode Hox proteins, *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*), and *abdominal-B* (*abd-B*) (Martin et al. 1995). The bulk of genomic DNA comprising the BX-C is thought to function as *cis*-regulatory DNA that controls the timing and site of Hox expression (Sanchez-Herrero et al. 1985). Nevertheless, it has been known for more than 15 years that intergenic regions of the BX-C are extensively transcribed (Cumberledge et al. 1990; Bae et al. 2002; Drewell et al. 2002). The possible functional activities of the noncoding RNAs have received little attention, despite the fact that these transcripts, including *iab-4*, are expressed in restricted domains along the anterior-posterior axis, like conventional Hox genes (Cumberledge et al. 1990).

Hox gene clusters contain conserved miRNAs. For ex-

ample, *miR-10* is located within the *Drosophila* Antennapedia gene complex (ANT-C) between the Hox genes *Deformed* and *Sex combs reduced* (Lagos-Quintana et al. 2001). The sequence and location of *miR-10* are conserved in vertebrate Hox complexes (Tanzer et al. 2005). *Sex combs reduced* has been proposed as a direct *miR-10* target in insects (Brennecke et al. 2005). A second group of *Drosophila* miRNAs map to a hairpin located at the distal end of the *iab-4* locus (Aravin et al. 2003), which resides between *abd-A* and *Abd-B* (Fig. 1A). miRNAs were cloned from both arms of this hairpin and are termed *iab-4-5p* and *iab-4-3p* (Aravin et al. 2003). *miR-iab-4-5p* was recently predicted to regulate *Ubx* activity (Stark et al. 2003; Grun et al. 2005). Although vertebrates lack an *iab-4* ortholog, as defined by sequence identity, a different miRNA, *miR-196*, resides at an analogous position adjacent to the posterior-most HOX 9–13 paralogs. Tissue culture assays, in vivo cleavage products, and transgenic lacZ “sensors” indicate that *miR-196* inhibits *Hoxb8* activity (Mansfield et al. 2004; Yekta et al. 2004). Despite these provocative target relationships, no phenotypes have been associated with any Hox miRNA.

miRNAs are short, 21–24-nt RNAs that attenuate protein synthesis by binding complementary sites in target mRNAs (Lai 2003; Bartel 2004). An unexpectedly modest amount of base-pairing appears to underlie target recognition. Experimental and computational studies have converged on the principle of “seed-pairing,” whereby ~7 continuous Watson-Crick base pairs at the 5'-end of the miRNA mediate target recognition (Lai 2002; Brennecke et al. 2005; Lewis et al. 2005). The limited sequence requirement for miRNA-mRNA interactions has fueled current proposals that a third or more of all mRNAs may be regulated by miRNAs (Lewis et al. 2005; Xie et al. 2005). As tallies of miRNA loci continue to grow (with current estimates for humans ranging from 800 to 1000) (Bentwich et al. 2005; Berezikov et al. 2005), the network of possible miRNA:target interactions will expand.

Only a small number of miRNA:target interactions have been studied in vivo. Here we present evidence that *iab-4* microRNAs selectively attenuate *Ubx* activity in vivo. The *Ubx* 3' untranslated region (3' UTR) contains predicted target sites for *miR-iab-4-5p* and expression of a *GFP-Ubx-3' UTR* “sensor” transgene is repressed by ectopic expression of a *mir-iab-4* minigene. This minigene also reduces *Ubx* protein levels in haltere imaginal discs, thereby inducing a classical homeotic transformation of halteres into wings. Taken together, these results suggest that the *iab-4* transcription unit encodes an authentic homeotic regulatory gene. We suggest that additional noncoding RNAs correspond to “missing” homeotic genes in the Bithorax complex, and discuss novel mechanisms of *iab-4* regulation during development.

Results and Discussion

Complementary patterns of *iab-4* and *Ubx* expression

Among the sequenced *Drosophilids*, there are only a few highly conserved sequences in the 120-kb region separating *abd-A* and *Abd-B* in the BX-C (Fig. 1A). Three are located in regions that flank known insulator elements (Karch et al. 1994; Zhou et al. 1996; Barges et al. 2000). A fourth conserved region is located within the 3' region of the

[Keywords: microRNA; *iab-4*; Ultrabithorax; homeotic gene]

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Article and publication are at <http://www.genesdev.org/cgi/doi/10.1101/gad.1372505>.

Figure 1. Structure, conservation, and expression of the *iab-4* miRNA. (A) A 120-kb interval of the *D. melanogaster* Bithorax Complex that includes the homeobox genes *abd-A* and *Abd-B*, the noncoding RNA gene *iab-4*, and three transcribed boundary elements (MCP, Fab-7, and Fab-8). Shown are VISTA plots depicting highly conserved sequences in the distantly related *Drosophila mojavensis* (regions of >80% identity in a window of 50 nt) and the honeybee *A. mellifera* (regions of >80% identity in a window of 150 nt). Note that all of these conserved regions are associated with transcribed noncoding elements. Depicted at higher magnification are two *iab-4* splice forms, an ~2.1-kb transcript containing the *iab-4* miRNA hairpin and a shorter transcript that terminates just 5' of the miRNAs. The two *iab-4* probes used in this study (*iab-4* probe1 and *iab-4* probe2) are shown as black bars below the schematics in A. (B) Perfect conservation of the *iab-4*-5p and *iab-4*-3p miRNAs in diverse arthropods: *D. melanogaster* (*D. mel*), *D. pseudoobscura* (*D. pse*), *Bombyx mori* (*B. mor*), *A. mellifera* (*A. mel*), *A. aegypti* (*A. egypt*), *A. gambiae* (*A. gam*), and *T. castaneum* (*T. cas*). (C–E) Time course of *iab-4* (red) and Ubx (green) nascent transcription: stage 5 (C), stage 8 (D), stage 11 (E). Nascent *iab-4* transcription was detected with an intronic probe marked as probe 1 in A. (F–H) Complementary expression of *iab-4* RNA (red) and Ubx protein (green) in a stage 12 embryo. Shown is a maximum projection through a sagittal section revealing the ectoderm and underlying mesoderm (approximately five cell layers). Domains of highest Ubx protein levels do not express *iab-4* (arrowheads), whereas cells with highest levels of *iab-4* do not accumulate Ubx (arrows). Other regions show low levels of coexpression (asterisk).

iab-4 transcription unit (Cumberledge et al. 1990; summarized in Fig. 1A). The *iab-4* locus contains regulatory DNAs that control *abd-A* expression (Karch et al. 1990). It also produces two spliced, polyadenylated transcripts that differ by the presence or absence of the highly conserved 3' sequences (Fig. 1A). This region contains a single ~100-nucleotide (nt) pre-miRNA hairpin structure that encodes two stable miRNAs: *iab-4*-5p and *iab-4*-3p (Aravin et al. 2003).

The *pre-mir-iab-4* hairpin is conserved both in sequence and genomic location in the mosquitoes *Anopheles gambiae* and *Aedes aegypti*, the honeybee *Apis mellifera*, and the flour beetle *Tribolium castaneum* (Fig. 1B), species that last shared a common ancestor roughly 400 million years ago. In fact, it is by far the best conserved sequence in the *abd-A/Abd-B* interval among *Drosophila* and non-*Drosophila* genomes (Fig. 1A). It is notable that the sequences corresponding to the mature *iab-4*-5p and *iab-4*-3p miRNAs (each forming one arm of the hairpin) are perfectly conserved among this broad spectrum of insects.

Double-label RNA FISH and antibody staining was

used to determine the relative expression patterns of *iab-4* RNA and Ubx RNA/protein accumulation during embryonic development (Fig. 1C–E). The *iab-4* primary transcript is strongly expressed in the presumptive abdomen, mainly in the progenitors of the second (A2) through seventh (A7) segments (see Cumberledge et al. 1990). There is also a weak transient stripe of expression in anterior regions. Ubx RNA is distributed in a strong stripe in parasegment 6, but only low levels are seen in regions of the presumptive abdomen containing high levels of *iab-4* transcript. No Ubx protein is detected at this early stage, possibly due to the time required to transcribe the entire ~80-kb locus (Shermoen and O'Farrell 1991).

During the rapid phase of germband elongation, Ubx protein becomes detectable in the abdomen (Fig. 1D). At the conclusion of germband elongation, the Ubx and *iab-4* patterns are largely complementary in the dorsal ectoderm (Fig. 1E). During segmentation of the germband, the Ubx protein shows complex modulation in the dorsal ectoderm (Fig. 1F–H). There are three apparent levels of Ubx protein distribution in these regions: high, low, and none (Fig. 1F). We note an inverse correlation between the levels of Ubx protein and the sites of *iab-4* expression (Fig. 1G,H). The strongest expression of *iab-4* occurs in regions having the lowest accumulation of Ubx protein (Fig. 1F–H, arrows), whereas intermediate and low levels coincide with sites of diminished Ubx accumulation (Fig. 1F–H, asterisks); there is little or no *iab-4* expression in those cells containing the highest levels of Ubx protein (Fig. 1F–H, arrowheads). These observations are consistent with the possibility that Ubx protein synthesis might be modulated by one or both *iab-4* miRNAs. Direct support for this possibility stems from the analysis of a *GFP-Ubx* transgene containing the 3' UTR sequence from Ubx. This transgene displays slightly diminished expression in abdominal regions containing high levels of *iab-4* transcripts (data not shown; see below).

The Ubx 3' UTR is directly targeted by miR-*iab-4*-5p

Computational studies have identified the Ubx 3' UTR as a likely target of regulation by *iab-4*-5p (Stark et al. 2003; Grun et al. 2005). Of the seven potential sites identified by Stark and colleagues (Fig. 2A), five exhibit conserved and canonical seed pairing of six or more nucleotides (Fig. 2B). Of these, sites #3 and #6 are perfectly conserved among sequenced *Drosophilids* and have seeds of at least 7 nt, a length sufficient for efficient *in vivo* recognition by miRNAs (Brennecke et al. 2005; Lai et al. 2005); site #7 also has a 7-mer seed match that is conserved in some species (see below).

In current target-finding approaches, greater confidence is usually ascribed to those miRNA-binding sites that are conserved in the greatest number of analyzed species. Curiously, the putative *iab-4*-5p target sites with the lowest free energy are not necessarily the best conserved. Instead, there appear to be compensatory changes among different *iab-4*-5p-binding sites in individual Ubx 3' UTRs. For example, site #4 exhibits canonical 6-mer seed pairing in four species of *Drosophila*, but contains a G:U base pair in *Drosophila virilis* and a seed mismatch in *Drosophila mojavensis* and is likely nonfunctional in these two species. Conversely, site #7 is mispaired in *D. melanogaster* and *Drosophila yakuba*,

Figure 2. Identification and validation of iab-4-5p target sites in the *Ubx* 3' UTR. (A) Conserved regions of the *Ubx* 3' UTR revealed by VISTA plots between *D. melanogaster* and other Drosophilids (dark blue, *Drosophila simulans*; light blue, *D. ananassae*; white, *D. mojavensis*). All but one of seven putative iab-4-5p target sites (Stark et al. 2003) correspond to islands of conserved sequence (numbered arrows); free energies of predicted iab-4-5p:target duplexes are noted below. (B) Alignments and free energies of likely iab-4-5p target sites from six divergent Drosophilids: *D. melanogaster* (*D. mel*), *D. yakuba* (*D. yak*), *D. ananassae* (*D. ana*), *D. pseudoobscura* (*D. pse*), *D. virilis* (*D. vir*), and *D. mojavensis* (*D. moj*). Conserved *Ubx* 3' UTR sequences are shaded gray; nucleotides that pair with the iab-4-5p seed are shaded blue. Note apparent compensatory mutations between strong/weak sites in different species (in red, arrows). For example, site #4 is likely active in *D. melanogaster* but inactive in *D. mojavensis*, while site #7 probably inactive in *D. melanogaster* but active in *D. mojavensis*. (C–H) Evidence for direct recognition of the *Ubx* 3' UTR by iab-4-5p. Shown is expression of a *tub-GFP-Ubx* 3' UTR sensor transgene (green) in a background where a *UAS-DsRed-iab-4* transgene has been activated using *ptc-Gal4* (red). The *Ubx* sensor is specifically down-regulated in *mir-iab-4*-misexpressing cells marked by expression of DsRed. (F–H) Close-ups of the boxed region in E.

but is conserved as a strong 7-mer seed-paired site in *D. mojavensis*, *Drosophila pseudoobscura*, *Drosophila ananassae*, and *D. virilis*. These observations suggest that individual target sites may be evolutionarily labile, and in vivo regulation depends on the net complement of both high- and low-affinity sites contained in the target mRNA. These compensatory changes in strong and weak target sites are reminiscent of the evolution of individual Bicoid-binding sites in the *eve* stripe 2 enhancers present in divergent Drosophilids [Ludwig et al. 1998].

Direct evidence for *iab-4:Ubx* miRNA interactions was obtained using a *tub::GFP-Ubx* 3' UTR transgene (the "Ubx sensor"). This construct directs ubiquitous expression of the *GFP* coding sequence fused to the *Ubx* 3' UTR, and wing imaginal discs bearing the Ubx sensor

display relatively uniform expression of GFP. Ectopic expression of *UAS-DsRed* under the control of *ptc-Gal4*, which directs expression along the anterior-posterior border of the disc, has little or no effect on the distribution of GFP staining (Brennecke et al. 2005).

We next assayed the expression of the Ubx sensor in the presence of ectopic *iab-4* miRNAs. For this purpose, a transgene was created that contains *DsRed* and 400 base pairs (bp) from *iab-4* encompassing the entire 100-bp 3' hairpin sequence (*UAS-DsRed-iab-4*). As shown previously, transgenes of this type direct the expression of biologically active miRNAs in cells that are labeled by expression of DsRed (Stark et al. 2003). When driven by *ptc-Gal4* in wing imaginal discs, Ubx sensor levels were specifically diminished in those cells expressing the *iab-4* transgene (Fig. 2C–E). Detailed analysis of the *DsRed-iab-4* and *GFP-Ubx* expression profiles suggests that repression of the Ubx sensor by ectopic *iab-4* miRNA is dose-sensitive (Fig. 2F–H). These data constitute *in vivo* evidence that *iab-4* miRNAs specifically recognize target sequences in the *Ubx* 3' UTR and thereby attenuate Ubx protein synthesis.

Ectopic iab-4 inhibits endogenous Ubx and induces a Ubx-like homeotic transformation

Ubx protein is broadly distributed throughout the haltere imaginal disc, where it imposes haltere identity by repressing the expression of many genes that otherwise direct wing development (e.g., Weatherbee et al. 1998). This repression is very sensitive to Ubx levels, and consequently, even partial loss of *Ubx* function can transform halteres into wings (i.e., Fig. 4B, below). Haltere discs were examined for the accumulation of Ubx protein in the absence or presence of ectopic *iab-4* miRNAs. Ubx is detected at high levels in most of the cells of the presumptive pouch. Expression of DsRed alone using *bx-Gal4*, which is active in the presumptive dorsal region of the pouch, did not affect Ubx accumulation (Fig. 3A–E). In contrast, haltere discs expressing *UAS-DsRed-iab-4* under the control of *bx-Gal4* displayed strongly reduced levels of Ubx protein (Fig. 3F–J). Thus, as seen for the Ubx sensor in wing discs, ectopic *iab-4* miRNA inhibits accumulation of endogenous Ubx protein.

The effect of *iab-4* miRNA misexpression on adult haltere development was examined. The wild-type haltere contains small lightly pigmented sensilla but lacks the triple row of sensory bristles at the leading margin seen in wings (Fig. 4A). In contrast, halteres that developed from discs expressing *UAS-DsRed-iab-4* under the control of *bx-Gal4* or *scalloped-Gal4* are flattened and elongated in the proximal–distal axis, and exhibit an extensive row of sensory bristles at the leading margin (Fig. 4C,D). All of these phenotypes are strongly indicative of a classic haltere-to-wing homeotic transformation (Roch and Akam 2000).

The demonstration that miR-iab-4 represses the anterior Hox gene *Ubx* might be relevant to the phenomenon of “posterior prevalence” (Duboule and Dolle 1989). *Polycomb* mutant embryos have previously been observed to derepress Hox gene expression, resulting in broad misexpression of all Hox genes (e.g., Pirrotta 1997). Ultimately, ectopic expression of posterior Hox genes (e.g., Abd-B or Hox9–13) leads to the transcriptional repression of anterior Hox genes (e.g., *Ubx* or *Hox8* paralogs) (Choi et al. 2000). We observe that *Polycomb* mu-

Figure 3. Expression of *mir-iab-4* in the haltere disc reduces endogenous Ubx protein accumulation. (A–E) Normal expression of Ubx protein (green) in *bx-Gal4/Y; UAS-DsRed/+* haltere discs. (A) Ubx is detected throughout the pouch region of the haltere disc. (B) DsRed (red) marks *bx-Gal4* activity in the dorsal compartment of the presumptive haltere pouch. (C) Merge. (D,E) Close-ups of the region boxed in C. (F–H) *bx-Gal4/Y; UAS-DsRed-mir-iab-4* haltere disc exhibits reduced accumulation of Ubx protein (green) in the presence of ectopic *mir-iab-4* (red). (I,J) Close-ups of the region boxed in H.

tant embryos also derepress *iab-4* expression throughout the embryo (Fig. 5, cf. B and A). Therefore, misexpression of *iab-4* miRNAs may contribute to the repression of Ubx function observed in the *Polycomb* mutant background. Thus, posterior prevalence may arise from the dual utilization of protein-based/transcriptional mechanisms and miRNA-based/post-transcriptional mechanisms (Fig. 5C).

Regulation and function of *iab-4* miRNAs

The detailed analysis of intergenic transcripts in the *abd-A/Abd-B* interval suggests a “strand exclusion” model for *iab-4* regulation (summarized in Fig. 5F). The *iab-4* locus is unusual in that both strands are transcribed (Bae et al. 2002). However, each strand displays a distinct pattern of expression. The strand producing the *iab-4* pri-miRNA is broadly expressed in the A2–A7 region of the germband, while the other strand is expressed in A8 and A9 (e.g., Fig. 5D,E). Double RNA FISH assays suggest that the miR strand is initially expressed in A2–A8, but expression is lost in A8 as transcription of the other strand progresses from the *iab-8* domain. Perhaps transcription from one strand diminishes transcription from the other (Fig. 5F). Although the detailed mechanism may be different, these observations are evocative of the mutually exclusive expression of *Xist* and *Tsix* RNAs on mammalian X chromosomes (e.g., Rougeulle and Avner 2004). Additional target predictions for miR-*iab-4* imply that exclusion of *iab-4* expression from A8

might be important for stable accumulation of other potential *iab-4* target mRNAs, such as *Abd-B* (Enright et al. 2003; Stark et al. 2003).

Traditionally, recessive loss-of-function mutations are used to assess the *in vivo* activities of patterning genes. The principal argument for *iab-4:Ubx* interactions in development rests with the analysis of dominant gain-of-function phenotypes arising from the misexpression of miR-*iab-4* in the haltere imaginal disks (see Figs. 3, 4). The specificity of the resulting haltere-to-wing homeotic transformation correlates with reduced levels of Ubx protein accumulation specifically in the regions where miR-*iab-4* products are misexpressed. It is likely that there is redundancy in the transcriptional repression of *Ubx* by *Abd-A* and *Abd-B* products, and the inhibition of Ubx protein synthesis by *iab-4* miRNAs. Indeed, noncoding genes in the BX-C were mainly identified by dominant mutations such as chromosomal rearrangements (e.g., Lewis 1978). Therefore, misexpression assays may prove effective in analyzing the function of other Hox noncoding genes.

This study provides evidence that *iab-4* encodes a novel homeotic regulatory activity, which functions, at least in part, by producing miRNAs inhibiting *Ubx*. *iab-4* miRNAs may regulate additional target mRNAs. For example, computational analyses identify *homothorax* as another potential target of interest (Grun et al. 2005). *Homothorax* works in parallel with the Hox co-factor Extradenticle and various Hox proteins to control

Figure 4. Directed expression of *mir-iab-4* induces a homeotic transformation. (A) Wild-type haltere, which contains small lightly pigmented sensilla but lacks the triple row of sensory bristles seen in wings. (B) Haltere-to-wing transformation in a mild *Ubx* loss-of-function mutant background. Misexpression of *iab-4* miRNA hairpin in *bx-Gal4/Y; UAS-DsRed-mir-iab-4* (C) and *sd-Gal4, UAS-DsRed-mir-iab-4* (D) animals induces a similar haltere-to-wing transformation.

Figure 5. Regulation and function of *iab-4*. (A,B) Expression of *iab-4* in wild-type (A) and *Pc*¹ (B) stage 12 embryos. The domain of *iab-4* transcription is expanded anteriorly in the *Pc* mutant, which correlates with suppression of *Ubx* activity in this mutant background. (C) Similar regulatory and functional arrangement of the BX-C and the vertebrate HOX clusters. Posteriorly expressed Hox proteins repress the transcription of anterior Hox genes; posteriorly expressed microRNAs (*iab-4-5p* and *iab-4-3p* in insects, and miR-196 in vertebrates) repress anterior Hox genes at a post-transcriptional level (either by transcript cleavage or inhibition of productive translation). (D,E) Double RNA FISH staining to localize *iab-4* transcripts (red) and a noncoding RNA produced from the opposite strand (blue). The opposite transcript is not detected with *iab-4* probes until the onset of germband elongation, presumably due to the time it takes for Pol II to progress from the *iab-8* promoter located ~75 kb away. In germband-elongated embryos the two transcripts are expressed in complementary patterns. The probes used for hybridization reside within 2 kb of one another within the *iab-4* transcription unit and are described in Figure 1. (F) Summary of the strand-exclusion model. A large transcript derived from the *Abd-B*/*iab-8* domain might preclude transcription from the *iab-4* promoter on the opposite strand.

the patterning of legs and antennae (for review, see Mann and Morata 2000). It is also possible that downstream transcriptional targets of *Ubx* ("realizators") might be modulated by *iab-4* miRNAs. Additional noncoding RNAs in the BX-C, such as *cbx*, *pbx* and *bx*d (e.g., Lipshitz et al. 1987), might also possess homeotic regulatory activities and account for the remaining genes identified by Lewis (1978).

Materials and methods

Immunohistochemistry and in situ hybridization

Embryo fixation, probe labeling, and nascent transcript RNA FISH were performed according to Kosman et al. (2004) with the following modifications. The sequence of the *iab-4* probes were previously described (Bae et al. 2002). RNA probes labeled with FITC-, DIG-, and Biotin-conjugated UTP were detected as follows: DIG, sheep α DIG (Roche); Biotin, mouse α Biotin (Roche); FITC, rabbit α FITC (Molecular Probes). The secondary detection was as follows: donkey α sheep Alexa Fluor 555 (Molecular Probes), donkey α mouse Alexa Fluor 488 (Molecular Probes), and chicken α rabbit Alexa Fluor 647 (Molecular Probes). *Ubx* protein was detected using mouse α -*Ubx* (FP3.83) (White and Wilcox 1984). For de-

tection of GFP and *Ubx*, we fixed discs in 4% paraformaldehyde and probed them with rabbit α -GFP (1:1000; Molecular Probes) or mouse α -*Ubx* FP3.83 (1:10).

Drosophila strains

The *UAS-miR-iab4* construct was generated by cloning a 400-bp fragment centered around the *mir-iab-4* hairpin downstream of pUAST-DSred2 (Stark et al. 2003). The *Ubx* sensor was cloned by inserting genomic DNA including the *Ubx* 3' UTR and 400 bp of downstream genomic DNA into *tub-GFP* (Brennecke et al. 2003). These constructs were injected into *w*¹¹¹⁸ embryos, and multiple transgenic lines were established. Other *Drosophila* lines were obtained from the Bloomington Stock Center, including *scalloped-GAL4*, *bx*^{MS1096}-*GAL4*, and various *Ubx* and *Pc* alleles.

Acknowledgments

We acknowledge Nipam Patel for antibodies, David Bilder for fly stocks, and Gerald Rubin for his support during this project. We also thank Robert Zinnen and Kimberly Mace for discussions and helpful comments on the manuscript, and Oliver Zill who provided important help during the initial phases of this work. This study was funded by grants from the NIH (GM34431 to M.L. and GM72395 to M.R.), and grants from the Leukemia and Lymphoma Society (LLS #3096-05) and the Burroughs Wellcome Foundation (CABS 1004721) to E.C.L.

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Genes Dev. 2005, **19**:

Access the most recent version at doi:[10.1101/gad.1372505](https://doi.org/10.1101/gad.1372505)

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