Rasl-Mediated Modulation of Drosophila Homeotic Function in Cell and Segment Identity

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ABSTRACT

Mutations of the Drosophila homeotic proboscipedia gene (pb, the Hox-A2/B2 homologue) provoke dose-sensitive defects. These were used to search for dose-sensitive dominant modifiers of pb function. Two identified interacting genes were the proto-oncogene RasL and its functional antagonist GapL, prominent intermediaries in known signal transduction pathways. RasL is a positive modifier of pb activity both in normal and ectopic cell contexts, while the RasL-antagonist GapL has an opposite effect. A general role for RasL in homeotic function is likely, since RasL activity also modulates functions of the homeotic loci Sex combs reduced and Ultrabithorax. Our data suggest that the modulation occurs by a mechanism independent of transcriptional control of the homeotic loci themselves, or of the RasL/GapL genes. Taken together our data support a role for RasL-mediated cell signaling in the homeotic control of segmental differentiation.

Making a fruit fly requires crucial contributions from homeotic "selector" genes. These selector genes direct the development of the structures unique to each segment: legs, wings, halteres, mouthparts. The mutation of a homeotic locus leads to the replacement, sometimes spectacular, of one body part by an inappropriate one. The homeodomain-containing transcription factors encoded by the homeotic genes regulate the expression of groups of target "realisator" genes that confer unique identities to the segmental units composing the embryonic and adult body (Lawrence and Morata 1994). The majority of known homeotic loci in Drosophila melanogaster are located in the Antennapedia and Bithorax Complexes and correspond to vertebrate homologues making up the Hox complexes (Kenyon 1994). In light of the striking evolutionary conservation of the homeotic/Hox genes and complexes, it is believed that these genes' functions are also highly conserved. This presumption, when tested, has been borne out (Malicki et al. 1990; McGinnis et al. 1990; Zhao et al. 1993; Poppier et al. 1995).

Homeotic gene functions are required in specific regions of the embryo, larva, and pupa as seen by the localized effect of loss-of-function mutations. Gene expression is generally found to be spatially restricted to the region requiring homeotic function. Selector function is clearly seen through the action of homeotic gain-of-function mutations that direct the formation of normal structures in inappropriate localities. Proper gene function leads to the formation of a complex, differentiated structure comprising numerous cell types correctly proportioned and positioned. Ectopic expression may lead to the fabrication of the same structure elsewhere.

How does the function of a single gene direct the development of a complex structure? Much of the literature concerning homeotic gene function has focused on their selector functions via transcriptional control in the cell nucleus (Botas 1993; Gehring et al. 1994). Several studies in mosaic animals concluded that homeotic function is cell autonomous: the action of these transcriptional regulators in a cell depends only on the genotype of that cell (Garcia-Bellido and Lewis 1976; Morata et al. 1983; Merrill et al. 1987). Still, this emphasis on the hierarchical regulation of downstream target genes, and on elements of protein structure (notably the homeodomain) involved in such regulation, is likely incomplete given the complexity of structures such as legs and wings formed through homeotic control. A small number of observations support a role for cell-cell communication in homeotic function. For example, one direct transcriptional target of the homeotic Ultrabithorax (Ubx) locus is the decapentaplegic (dpp) gene encoding a conserved TGF-β related growth factor (Capovilla et al. 1994). Ubx activates dpp expression in the visceral mesoderm (Immergluck et al. 1990): the secreted DPP protein then modulates activity of the homeotic labial gene in cells of the adjacent endoderm (Immergluck et al. 1990). Thus at least one homeotic gene, Ubx, can send a signal and one other, labial, is capable of responding to it. Second, nonautonomous behavior of mitotic Antp+ clones in mosaic animals was reported a number of years ago (Struhl 1981). This observed nonautonomy suggests intercellular communication within the imaginal disc cells generating the appendage.

We present evidence here that homeotic function is
modulated by functions of the proto-oncogene Ras1, likely by cell signaling pathways. Our principal model is the proboscipedia locus (pb: homologous to Hox-A2/B2), required for adult mouthparts development. In the absence of pb function, the adult labial palps are transformed to prothoracic legs and the maxillary palps reduced to vestigial stubs (KAUFMAN 1978; PULTZ et al. 1988; CRIBBS et al. 1992). The homeotic gain-of-function pb phenotype is a transformation of the adult antennae to maxillary palps (CRIBBS et al. 1995). Both the loss- and gain-of-function transformations are dose-sensitive. In searching for dose-sensitive modifiers of pb function, we found that altering activity of the proto-oncogene Ras1 or of its antagonist Gap1 can lead to changes in the attribution of specific cell identities within a segment, or of segmental identity. Ras1 is centrally involved in the signal transduction pathways passing by the sevenless and torso receptor tyrosine kinases, modifying the nuclear activity of transcription factors via the balance of RAS1-GTP (active form) and RAS1-GDP (inactive form) (PERRIMON 1994; SIMON 1994).

We present here the first evidence for a functional link between the Ras1 proto-oncogene and homeotic function. The observed modulation of homeotic activity by Ras1 is likely to be general, since we find that homeotic activities of the Ubx and Sex combs reduced (Scr) loci are also sensitive to Ras1 activity levels.

MATERIALS AND METHODS

Fly culture and phenotypic analysis: All stocks and crosses were maintained at 25° on standard yeast-agar-cornmeal molas- ses medium. Phenotypes were initially regarded under a ste- reomicroscope; detailed analyses were performed by light mi-

microscopy (Zeiss Axiopt) after mounting dissected samples in Hoyer’s medium or by scanning electron microscopy.

Deficiency screen: The collections of deficiency stocks for the second and third chromosomes (DK2 and DK3) were obtained from the Indiana University Drosophila Stock Cen-
ter (IUDSC), Bloomington, Indiana. Females heterozygous for a given deficiency were mated with males carrying the HSPB:4d line, carrying a chimeric gene composed of the Hsp70 promoter fused to an 8.6-kb genomic duplication Dp(3;3)M-S31-2, in the heterozygous state such chromosomal deficiencies remove Ras1, required for adult development, we sought dominant mutations that synergistically modify HSPB developmental activity. This was accomplished by testing HSPB lines in combi-

nation with deletion mutations removing defined portions of the genome. In the heterozygous state such deletions reduce by about half the activity of all genes within that interval, but show no visible developmental defects. We sought deletions whose combination with HSPB modified the antenna-to-maxillary transforma-
tion, or led to novel phenotypes provoked neither by HSPB alone, nor by the heterozygous deletion. Among 110 autosomal deficiencies tested (representing about half the genome), 10 showed dose-sensitive interactions

RESULTS

Specific functional interactions at the molecular level in vivo have in many cases first been identified as dose-

sensitive genetic interactions. Loss-of-function proboscipedia (pb) mutations can yield qualitatively distinct adult transformations (KAUFMAN 1978; PULTZ et al. 1988; CRIBBS et al. 1992). In the pb+ condition, the adult mouthparts comprise normal labial palps. Partial loss-of-function leads to the partial replacement of labial tissue by aristae, the plumed distal antennal structures. The pb null condition results in complete replacement of the labial palps by prothoracic legs. Certain hypomorphetic alleles show a marked dosage sensitivity, allowing for transformations of labium to antenna, or to leg, in closely related conditions. Conversely, gain-of-function achieved by ectopic PB expression in transgenic HSPB lines (carrying a chimeric gene composed of the Hsp70 promoter fused to an 8.6-kb pb mini-gene) leads to the transformation of antennae to maxillary palps (CRIBBS et al. 1995). As for the action of loss-of-function mutations on the labial palps, this homeotic transformation of the antennae is dose sensitive. A highly reproducible partial transformation of antennae to maxillary palps results from basal (uninduced) expression of a single HSPB copy. Two copies result in a nearly complete antenna-to-maxillary transforma-
tion. Similarly, dose-sensitive effects due to the ectopic expression of PB protein from HSPB are also detected in the wings, the eyes, the posterior head and the prothoracic legs.

To identify genes that interact with pb in directing normal development, we sought dominant mutations that synergistically modify HSPB developmental activity. This was accomplished by testing HSPB lines in combi-

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interacts with HSPB in controlling certain cell identities. In contrast, we did not detect a reliable reduction in the HSPB-induced Ant to Mx transformation with RasI^{de} or RasI^{null}. We thus cannot formally exclude the possibility that a second gene in this interval also interacts with pb.

A very weak modification of the wings was detected in animals carrying HSPB in heterozygous combination with Df(3L)AC1, a deletion removing the interval 67A2; 67D11-13. This region contains the Gapl gene encoding a functional antagonist of RasI signaling. GAP1 protein activates the GTPase activity of RAS1-GTP, favoring conversion to the inactive GDP-bound form (Gaul et al. 1992). Having confirmed the phenotypic interaction between HSPB and RasI, the effects of GapI mutations on HSPB were examined. The allele employed, GapI^{Pc1}, is a P insertion described as a strong or null mutation (Rogge et al. 1992) that we refer to hereafter as GapI^{−}.

Heterozygous combinations of GapI^{−} with HSPB gave only a very weak effect. Importantly, though, the GapI^{−} HSPB/GapI^{−} genotype led to a marked enhancement of the Ant to Mx transformation (Figure 2D). Since GapI is generally viewed as a specific antagonist of RasI^{−} function, this supported our interpretation that RasI is the relevant locus in the 85D11-14 interval that modifies both homeotic segmental transformation and wing vein formation. We therefore compared the Ant to Mx transformation in GapI^{−} HSPB/GapI^{−} adults with GapI^{−} HSPB/GapI^{+} RasI^{+} (Figure 2, D and E). Among the adults eclosing in this sensitized context, we observed a reduction of the Ant to Mx transformation. This reduction due to a RasI point mutation confirms that both RasI and GapI mutations can modify the segmen-

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**Figure 1.** Autosomal intervals containing dose-sensitive modifiers of HSPB. Chromosomes 2 and 3 are represented by the heavy lines, with a black oval for the centromere. Indicated above the lines are the names and extents of the defect intervals, with Df(SL)AC1, a deletion removing the interval 67A2; 67D11-13. This region contains the Gapl gene encoding a functional antagonist of RasI signaling. GAP1 protein activates the GTPase activity of RAS1-GTP, favoring conversion to the inactive GDP-bound form (Gaul et al. 1992). Having confirmed the phenotypic interaction between HSPB and RasI, the effects of GapI mutations on HSPB were examined. The allele employed, GapI^{Pc1}, is a P insertion described as a strong or null mutation (Rogge et al. 1992) that we refer to hereafter as GapI^{−}.

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Mutations of Ras1 and Gap1 modify the HSPB-induced antenna-to-maxillary palps transformation. (A) Wild-type antenna comprising, from proximal to distal, the antennal segments A1, A2, A3, A4 and the distal arista (ar). (B) An antenna of a HSPB:4d/+ fly showing a partial transformation of the antennal segment A3 (Mx) and of A4 and the arista (open arrowhead) toward maxillary palps (Ant to Mx). (C) An antenna of a HSPB/Df(3R)by-416 (Ras1+/+) fly. The deletion of one Ras1 copy leads to a partial reversion of the homeotic Ant to Mx transformation indicated by A3 and the arrowhead pointing to A4. (D) An antenna of a Gap1—HSPB/Gap1— fly. The transformation of antenna toward maxillary palps is more complete in the presence of the Gap1 mutation, as seen by the reduction of the arista and its replacement by a more horizontal maxillary palp (Mx). The effect of the homozygous Gap1— condition on the HSPB phenotype is opposite to that of a heterozygous deletion of the Ras1 interval in C. (E) Antenna of Gap1— HSPB/Gap1— Ras1/ fly. The heterozygous presence of a Ras1 point mutation reduces the Gap1—conferred enhancement of the HSPB-induced Ant to Mx transformation.

tal transformation that results from ectopic PB expression. Further, Gap1— homozygotes possess additional wing vein tissue (Figure 3D), a defect aggravated by the presence of HSPB (Gap1— HSPB/Gap1—; see Figure 3E). This mutant phenotype was reduced by a Ras1 point mutation (Gap1— HSPB/Gap1— Ras1/; see Figure 3F). These data support the interpretation that the Ras1 locus modifies ectopic pb homeotic activities in both segmental transformation and wing vein formation. We note, however, that this interpretation is based on our explicit (and as yet unproven) presumption that pathways with common components are employed in both wing and antennal development. As shown in the next section, the sensitive genetic interactions identified in the antennae and wings reflect interactions that can also be detected in the mouthparts, the normal site of pb function.

Ras1 function modulates normal pb activity in the mouthparts: Although the modified wings and the Ant to Max homeotic transformation offer sensitized contexts to screen for genetic interactors with pb, neither is a normal site for pb function. We therefore examined whether similar effects for Ras1 and Gap1 could be detected in a normal lieu of pb function. Double mutant combinations were constructed placing appropriate pb hypomorphic mutations in combination with either the null allele Ras1C06 (Ras1—) or with Gap1—. One test employed the pb alleles pb/ and pb/; an
FIGURE 3.—HSPB interacts with RasI and Gap1 in the wings. (A) Wild-type wing. The longitudinal veins L2, L3, L4 and L5 and the posterior crossvein between L4 and L5 (cv) are indicated. (B) A HSPB/+ wing. Most of these wings are wild type, but a small proportion (~10% of female wings and ~2% for males) possess an ectopic bristle on the distal end of the vein L3 (arrow and inset enlargement). (C) Wing of a HSPB/Ras1^18 fly. Whereas HSPB/+ and Ras1^18/+ wings are nearly wild type, their combination provokes a partially penetrant truncation of wing veins L4 (and L5, not shown) and augments the frequency of the ectopic L3 bristle (~90% for females and ~50% for males; see arrow and inset enlargement). (D) A Gap1^- wing showing ectopic vein tissue near the junction of L2 with the wing margin, thickening of the L3 vein extremity (open arrowhead), and modification of the crossvein. (E) Wing of a Gap1^-HSPB/Gap1^- fly. The HSPB transgene enhances the effects of Gap1, yielding ectopic veins that are more prominent, particularly adjacent to the distal end of L3 (closed arrowhead). (F) Wing of a Gap1^-HSPB/Gap1^- Ras1^18 fly. As for the Ant to Mx transformation (Figure 1E), the Ras1^18 mutation diminishes the effects of Gap1 on HSPB-induced phenotypes in the wing.

intermediate strength hypomorph and a protein null, respectively. We compared pb^3 Ras1^C608/pb^4 and pb^3 Ras1^+ /pb^4 animals. The pb^3/pb^4 combination leads to a mixed transformation of the distal labium to leg/antennal appendages. The prothoracic or T1 leg tissue can be distinguished from labial or arista tissue by the appearance of leg-specific bracted bristles, distal claws and associated sense organs, and the male-specific sex comb. On examining adult pb^3 /pb^4 males with two (control) or only one functional copy of Ras1^+, the labial palps of the latter showed generally more severe mutant phenotypes including the appearance of prothorax-specific sex comb teeth and distal claws (Figure 4, A and B). These results thus clearly support a role for Ras1^+ activity in wild-type pb homeotic function in the adult mouthparts.

Adults homozygous for pb^4 and Gap1^- pb^4 were also examined in the hypothesis that the Gap1^- condition would lead to increased pb^- activity. The pb^- genotype leads to a reliable partial transformation of the labial palps to antennal aristae (Figure 4C). In the Gap1^- pb^4 double mutant the labial to antennal transformation was consistently altered toward wild type (Figure 4D). This indicates that reduced Gap1^- activity augments pb^- function in the distal labium.

Taken together these data indicate that Ras1^-Gap1 functions modulate pb^- activity in a variety of cell types, including the mouthparts and the antennae. Ras1^- acts as a positive modulator of pb^- activity, and Gap1^- exerts an opposite effect. This is true for gain-of-function PB phenotypes in diverse contexts including the antennae (Figure 2), wings (Figure 3) and legs (not shown). Importantly, it is also true for normal pb^- functions in the mouthparts (Figure 4).

Ras1^- activity modulates the homeotic activities of Scr and Ubx: To test whether the modification of pb^- homeotic activity by Ras1^- might be more general, we examined the functional relationship between Ras1^- and the homeotic Sex combs reduced (Scr) and Ultrabithorax (Ubx) loci.
Ser: Normal male flies carry a sex comb on the most proximal tarsal segment of the prothoracic (T1) leg, composed of "teeth" that are specialized bristles. Prothoracic identity, including the presence of the single, properly placed sex comb, depends on the homeotic Sex combs reduced locus (Pattatucci and Kaufman 1991; Pattatucci et al. 1991). Ser+ function is haploinsufficient (hence dose-sensitive), as most simply visualized by the sex comb. Whereas normal males possess a sex comb with ~12 teeth, in Ser-/Ser- heterozygotes this comb is reduced to approximately six teeth (Figure 5A). Because quantitative modulations of the sex comb were not readily detected on modifying Ras1 activity, we sought a more sensitized background in which to examine potential interactions of Ser with Ras1.

Another locus affecting sex comb formation is sex combs distal (scd), an X-linked gene represented by the single viable mutant allele (Lindsley and Zimm 1992). About 70% of mutant scd; Ser- males carry a small distal sex comb on the second tarsal segment of the T1 leg (Figure 5C). Reducing Ser- function by half in scd; Ser-/Ser- males abolishes the more distal sex comb (though the single remaining sex comb contains approximately eight teeth instead of six; Figure 5B). Conversely, increasing Ser+ function by introduction of the chromosomal duplication Dp(Y;3)Antp+ leads to the fully penetrant appearance of a second sex comb containing on average four teeth (Figure 5D). Dp(Y;3)Antp+ comprises a duplication of the entire Antennapedia Complex, but the observed enhancement is attributable to Ser+ alone since it is reversed by an Ser point mutation (not shown). An effect similar to that of the duplication was obtained with the gain-of-function allele Ser+scd (not shown). These data show that the fabrication of a second more distal sex comb depends on Ser+ function.

The effects of Ras1/Gap1 activities on the formation of a distal sex comb were then examined. As for Ser+, scd males with increased dosage of Ras1+ showed a prominent second sex comb with full penetrance, as seen for scd; Ras1+/Dp(3;3)M-S31-2 (carrying three functional copies of Ras1; Figure 5F). The same effect is observed on reducing activity of the Ras1 antagonist Gap1+, as seen for scd; Gap1- (Figure 5F). The effect of Dp(3;3)M-S31-2 containing a supplementary Ras1+ copy is reversed when placed in combination with the Ras1 point mutation Ras1-1b (not shown), and is thus specifically attributable to Ras1+. The formation of a more distal sex comb depends on the state of both Ser+ and Ras1+ activities.

Ubx: Normal haltere development is sensitive to Ubx
Figure 5.—Interaction between Sex combs reduced (Scr) and Ras1/Gap1. These tests were carried out in a sensitized genetic context that yields an enhanced phenotypic effect, by placing all mutant combinations in a background carrying the X chromosome-linked sex combs distal (scd) mutation. (A) The prothoracic or T1 leg of an Scr+/+ adult male. The sex comb on the first tarsal segment is reduced by about half by the haploinsufficient Scr null mutation. (B) The T1 leg of an scd; Scr+/+ male. The scd allele partially restores the sex comb teeth removed in the Scr+/+ phenotype. (C) The T1 leg of an scd; +/- male fly. The scd mutation provokes a small, more distal sex comb on the second tarsal segment. This comb contains two teeth on average, and is detected in ~70% of adult males. (D) The T1 leg of scd; T(Y;3)Antp+/+ fly. The scd phenotype is enhanced (penetrance is raised from 70 to 100%, and the number of ectopic sex comb teeth is increased from two to about four) by a duplication of the Antennapedia Complex (Antp). This effect is due to increased Scr locus activity alone since it is reversed by placing the duplication in combination with the Scr' point mutation. (E) The T1 leg of an scd; Dp(3;3)M-S1-2(Dp(Ras1))/+ male. The duplication of the Ras1' gene enhances the distal sex comb phenotype. (F) A T1 leg of a scd; Gap1+/-male. The reduction of Gap1 function has the same effect on the scd phenotype as does increasing Ras1' function by a duplication. As for Scr, the effect of the chromosomal duplication is reversed by a point mutation of the Ras1 locus.

Figure 6.—The haploinsufficient Ubx haltere to wing transformation is modified by the Gap1'-condition. (A) A wild-type haltere. (B) A haltere from a Ubx+/+ fly. The Ubx haploinsufficient phenotype described as a partial haltere to wing transformation is characterized by the appearance of bristles (indicated with arrow) forming and apparent anterior wing margin on the enlarged appendage. (C) Haltere of Gap1'Ubx+/Gap1' fly. The Gap1' mutant enhances the haploinsufficient Ubx loss of function phenotype obtained with this Ubx null allele, with additional wing margin bristles (including more proximally) on the further enlarged appendage. All images are magnified 250×.

dose, as shown by the haploinsufficient transformation toward wing in Ubx-/-Ubx- animals (Figure 6B). In contrast to HSPB, we did not detect dose-sensitive (hetero-
zygous) interactions between Ubx and the RasI deletion chromosomes Df(3R)by-10 and Df(3R)by-116, nor with the Gap1 deletion chromosome Df(3L)AG-1. However, the haploinsufficient haltere to wing phenotype of Ubx<sup>109/+</sup> was clearly aggravated in Gap1<sup>-</sup> Ubx<sup>109</sup>/Gap1<sup>+</sup> adults (Figure 6, B and C). This effect of Gap1 on Ubx function is partially reversed in Gap1<sup>-</sup> Ubx<sup>109</sup>/Gap1<sup>+</sup> Ras<sup>16</sup> individuals (not shown), again supporting mutually antagonistic roles for Ras1 and Gap1 activities in Ubx function. Interestingly, this observation suggests that Gap1<sup>+</sup> interacts oppositely with Ubx compared to pb and Scr (above), since Ubx<sup>+</sup> function is favored rather than opposed by Gap1<sup>+</sup> activity.

**Ras1/Gap1 modulation of pb homeotic activity and transcriptional regulation of these genes:** Ras1<sup>+</sup> is formally an activator of pb while Gap1<sup>+</sup> is a negative modulator of pb<sup>+</sup> function in adult development. If the observed effect occurs at the level of pb transcriptional regulation, altering Ras1 or Gap1 activity should alter pb expression. We examined PB accumulation in the labial discs of homozygous Gap1<sup>-</sup> third instar larvae (from homozygous Gap1<sup>-</sup> mothers) by immunostaining with anti-PB sera. PB expression appeared normal in Gap1<sup>-</sup> embryos (not shown) and in the larval labial imaginal discs (Figure 7, A and B). Spatial expression of pb appears normal, as does PB accumulation within expressing cells. These data argue against a role of Gap1 in modulating pb activity via transcriptional regulation.

We were unable to examine PB expression in Ras1<sup>-</sup> imaginal discs, since Ras1<sup>-</sup> is embryonic-lethal and previous results indicate that the Ras1 condition is cell lethal in adult development (Simon et al. 1991).

We also tested the inverse hypothesis, that the PB homeodomain protein could regulate the transcription of the Ras1 and/or Gap1 genes. We therefore examined the expression of Ras1 and Gap1 mRNAs in labial discs from pb<sup>+</sup> and pb<sup>-</sup> larvae by in situ hybridization. No change of expression was detected for Gap1 in discs of mutant pb larvae (Figure 7, C and D). Ras1 expression was similarly indifferent to pb activity (data not shown).

Taken together, these results show that the activities of three homeotic selector loci can be altered by the activities of the Ras1 and Gap1 loci. Further, these observations suggest that the modulation occurs by a mechanism independent of transcriptional control, either of the homeotic loci themselves or of the Ras1/Gap1 genes.

**DISCUSSION**

Homeotic mutations can lead to the replacement of one body part by another. In some cases the transformation is dramatic, yielding flies with four wings instead of two or with legs in place of antennae. This remarkable capacity implies a mechanism that permits the coordination of homeotic gene action within a segment.
yielding many different cell types in correct numbers and distributions. In the present work we have found that homeotic activities can be modulated by Rasl-mediated signal transduction. We observe Rasl-modulated changes in homeotic effects on cell identity (bristle to distal sex combs, wing trichomes to veins, veins to trichomes or veins to bristles). Only a small number of cell identities in precise contexts are changed by HSPB activity. This suggests that most cells are aware of their positions and correct associated fates, perhaps as a consequence of cell-cell communication. We have also observed Rasl-dependent modifications of segmental identity. These occur in a concerted fashion on groups of adjacent cells, again suggesting cell communication.

Here we have found that a Rasl-mediated activity modulates homeotic function of the pb, Scr and Ubx loci in their normal contexts. Rasl acts as a positive modulator of pb and Scr, but as a negative modulator of Ubx in the halteres (as seen by reducing its inhibitor Gap1). Further, our data support the interpretation that transcriptional regulation of the homeotic genes is not involved. An artifactual "phenocopy" role of Rasl in cell proliferation leading to changes in cell differentiation can apparently be excluded. For example, the phenotypes associated with pb mutations in the mouthparts, or Scr mutations in the first leg, can be modified by Rasl/Gap1 mutations in a fashion that appears to affect attribution of specific cell identities, without changing appendage size (Figures 4 and 5).

Existing models of Rasl activity involve the transduction of external signals through membrane-bound receptors, across the molecular switch Rasl, and subsequently via protein kinase cascades to modify specific nuclear transcription factors. Rasl might modify pb activity through known signal transduction pathways, employing protein kinases and phosphatases to modulate activity of the PB protein. Consistent with this possibility, the mutant phenotype in hypomorphic pb adults is ameliorated in a Gap1 context (Figure 4, C and D), whereas no change occurred on combining Gap1 with the protein null allele pb (not shown). Mutants of the known MAP kinase-associated protein kinases that act in Rasl-mediated signal transduction pathways were therefore tested for dose-sensitive interactions with HSPB. However, no modification of HSPB function was detected in combination with a deletion of the Sos gene encoding a nucleotide exchange factor (Simon et al. 1991) nor with mutations of the protein kinase genesraf (Dickson et al. 1996), Dsor (Tsuda et al. 1993), hemipterous (Glise et al. 1995), rolled or Sevenmaker (rolled) (Biggs et al. 1994).

Though Rasl can modify homeotic selector functions, we have been unable to find any evidence that would place this interaction within the framework of known signal transduction pathways (Perrimon 1994; Hunter 1995). The absence of detectable interactions between known Ras-associated protein kinases and HSPB leaves open the possibility that Rasl modifies homeotic function by a new and as yet unknown mechanism. We feel that this is unlikely since we were able to detect interactions of pb, Scr and Ubx with both Rasl and Gapl, and in each case Rasl and its antagonist Gapl acted oppositely. This supports the interpretation that Rasl activity modulates homeotic activity by signal transduction in a manner related to the presently known pathways.

If signal transduction provides the connection between Rasl/Gap1 and the homeotic functions, three potential explanations may rationalize the absence of a detectable interaction with known protein kinase genes. First, the conditions employed may simply have been insufficiently sensitive. [We note, however, that similar screens based on dose sensitivity readily revealed the MAP kinase gene rolled (Dickson et al. 1996)]. A second possibility is that signal transduction through Rasl is directed toward the homeotic genes via as yet unidentified protein kinase cascades. A third possibility, not necessarily exclusive of the second, is that the homeotic activity can be modified by multiple Rasl-mediated protein kinase cascades. This view is potentially satisfying in light of the role that signaling seems likely to play, namely in permitting the integrated development of diverse cell types composing an appendage. It is also worth noting in this light that while Rasl and Gap1 act oppositely on the homeotic functions examined here, they do not act with equal "weights." the effects of Rasl could generally be detected in heterozygotes, whereas for Gap1 clear effects were obtained only in the homozygous state.

An important problem will now be to identify putative protein kinases modifying homeotic gene function in vivo. Continued genetic screens for interacting loci will undoubtedly reveal new genes implicated in such pathways. Biochemical approaches should permit access to elements of this functional connection: for example, the use of site-directed mutagenesis to change homeotic protein coding sequences coupled with the establishment of transgenic lines, will permit functional tests of the hypothesis that the homeotic selector proteins themselves are the targets of protein kinases. Much further work will clearly be necessary to elucidate the molecular basis of the relations between Rasl-mediated signaling and homeotic function. Given the evolutionary conservation of these various molecules, these will be most interesting questions to address. The power of the genetic use of dose-sensitive modifiers in diverse genetic contexts should afford numerous means to address these questions, and the present work offers useful starting points in this direction.

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LITERATURE CITED


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