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mirror, a *Drosophila* homeobox gene in the *iroquois* complex, is required for sensory organ and alula formation

Benjamin T. Kehl[1], Kyung-Ok Cho[1] and Kwang-Wook Choi[1,2,*]

[1]Department of Cell Biology, [2]Department of Ophthalmology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

*Author for correspondence (e-mail: kchoi@bcm.tmc.edu)

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SUMMARY

The *Drosophila* notum, the dorsal body wall of the thorax, is subdivided genetically into longitudinal domains (Calleja, M., Moreno, E., Pelaz, S. and Morata, G. (1996) *Science* 274, 252-255). Two homeobox genes clustered in the *iroquois* complex, *araucan* and *caupolican*, regulate proneural genes and are required for development of sensory bristles in the lateral notum (Gomez-Skarmeta, J. L., del Corral, R. D., de la Calle-Mustienes, E., Ferres-Marco, D. and Modolell, J. (1996) *Cell* 85, 95-105). An *iroquois*-related homeobox gene, *mirror*, was recently isolated and is localized close to the *iroquois* complex region (McNeil, H., Yang, C.-H., Brodsky, M., Ungos, J. and Simon, M. A. (1997) *Genes and Development* 11, 1073-1082; this study). We show that *mirror* is required for the formation of the alula and a subset of sensory bristles in the lateral domain of the notum. Genetic analysis suggests that *mirror* and the other *iroquois* genes interact to form the alula as well as the sensory organs. Based on similarities between *mirror* and the *iroquois* genes in their genetic map positions, expression, protein structure and function, *mirror* is considered a new member of the *iroquois* complex and is involved in prepatterning sensory precursor cells in the lateral notum.

Key words: *Drosophila*, Homeobox, Alula, Sensory bristle, *iroquois* (*iro*), *achaete-scute* (*ac-sc*), *mirror* (*mirr*)

INTRODUCTION

The pattern of bristles on the *Drosophila* thorax is remarkably precise and has been used extensively as a model to study the genetic basis of pattern formation. The thorax is covered with 22 macrobristles, which can be easily identified by their unique positions. The macrobristles are sensory organs of the adult periphery nervous system. Each organ consists of one neuron and three non-neuronal cells, all of which originate from a single sensory organ precursor (SOP) cell (Hartenstein and Posakony, 1989). Thoracic structures are derived from the wing imaginal disc, which is compartmentalized into several subregions (Garcia-Bellido et al., 1973; Bryant, 1975; Williams et al., 1993). These specific regions give rise to the wing as well as other thoracic structures, such as the notum and pleura, which correspond to the dorsal and ventral body walls of the thorax, respectively. An interesting developmental question is how a cell in the notum region of the wing disc is defined to form a bristle sensory organ at a specific position, leading to a precise two-dimensional pattern of macrobristles in the adult thorax.

A number of mutations in *Drosophila* have been characterized that alter the pattern or number of sensory organs (Jan and Jan, 1990). These studies have suggested that the formation of sensory organs occurs in sequential steps (de la Concha et al., 1988; Hartenstein and Posakony, 1989; Huang et al., 1991). Selective groups of cells in the wing disc express the proneural genes *achaete-scute (ac-sc)*, which commit these cells to become proneural clusters (Garcia-Bellido, 1978; Villares and Cabrera, 1987; Ghysen and Dambly-Chaudiere, 1989; Jan and Jan, 1990). One cell is singled out from each proneural cluster to become a sensory organ precursor (SOP). The SOP then prevents neighboring cells from becoming SOPs by lateral inhibition (Simpson, 1990). The *ac-sc* genes are involved in the initial decision to differentiate a bristle, but not in the differentiation process itself. *ac* and *sc* are required for different subsets of bristles (Ghysen and Dambly-Chaudier, 1989), suggesting that the field of bristle formation might be further subdivided based on a prepattern as proposed by Stern (1954). Therefore, it is important to understand how the expression of proneural genes is controlled by a prepattern of transcriptional regulators in different regions of the epidermis.

Several genes that regulate *ac-sc* gene expression have been characterized. For example, *hairy (h)* and *extramacrochaete (emc)* act as negative regulators of *ac-sc* since mutations in these genes result in the generation of ectopic SOPs (Skeath and Carroll, 1991). Proteins encoded by these genes, as well as AC-SC, contain basic helix-loop-helix domains that were found in a number of proteins involved in transcriptional regulation (Villares and Cabrera, 1987; Murre et al., 1989; Rushlow et al., 1989; Ellis et al., 1990; Garrell and Modolell, 1990). Hairy was shown to be a direct transcriptional regulator of *ac-sc*, while Emc appears to down-regulate *ac-sc* indirectly by interacting with other factors (Gomez-Skarmaeta et al.,

1995). Pannier (Pnr), a zinc finger protein with homology to the vertebrate transcription factor GATA-1, also acts as a negative regulator of *ac-sc* (Ramain et al., 1993; Heitzler et al., 1996). The *u-shaped (ush)* gene is involved in transregulation of *ac-sc* in the dorsal region of the notum (Cubadda et al., 1997). Ush, a zinc finger protein, heterodimerizes with Pnr as a cofactor and negatively regulates the transcriptional activity of Pnr (Haelin et al., 1997).

iroquois (iro) has been recently identified as a candidate for prepattern genes (Dambly-Chaudiere and Leyns, 1992; Leyns et al., 1996; Gomez-Skarmeta et al., 1996; Gomez-Skarmeta and Modolell, 1996). In contrast to *pan* and *ush*, which affect the dorsal notum, *iro* mutations specifically eliminate bristles in the most lateral domain in the notum without affecting bristles in the dorsal region (Dambly-Chaudiere and Leyns, 1992; Leyns et al., 1996; Calleja et al., 1996). The lack of lateral bristles in these mutants is due to the loss of *sc* expression, resulting in the failure of SOP formation (Leyns et al., 1996). The specific effects of *iro* mutations in the lateral notum suggest that the notum is divided into subdomains that can be genetically distinguishable. Indeed, studies on the pattern of gene expression in adult structures have provided evidence that the thorax is divided into previously unsuspected genetic subdomains (Calleja et al., 1996). The notum can be subdivided into at least three longitudinal dorsal bands along the body length: a central region, a lateral region and a region that falls between the central and lateral domains. Genes expressed in these longitudinal domains may be responsible for the establishment of the prepattern of individual pattern elements, such as bristles (Calleja et al., 1996).

Two clustered genes isolated from the *iroquois* region, *araucan (ara)* and *caupolican (caup)*, encode similar homeobox proteins. These genes also show similar spatial expression and function in the wing. Because of their structural and functional similarities, these loci were named the *iroquois* complex (IROC) (Gomez-Skarmeta et al., 1996). Confirming the function of *iro* genes in the lateral notum, both *ara* and *caup* are expressed in the presumptive lateral heminotum of the wing disc (Gomez-Skarmeta et al., 1996). The ARA protein was shown to directly bind to an *ac-sc* enhancer element. Therefore, the *iro* genes fulfill the characteristics of prepattern genes that direct sensory organ formation in the notum.

Such regional control of gene expression can also be found in other tissues. Enhancer trap strains containing a *P-lacW* element have been isolated that show specific silencing of the *white* gene expression in the ventral domain of the eye (Sun et al., 1995; Brodsky and Steller, 1996; Choi et al., 1996; McNeil et al., 1997). Such strains are attractive since genes nearby the *P-lacW* insertions might also be expressed in either dorsal- or ventral-specific regions and therefore may be involved in dorsoventral patterning of the eye. Interestingly, most of these strains contained the *P-lacW* insertions in the vicinity of the *iroquois* genes. One of these genes, *mirror* (*mirr*), which is expressed in the dorsal half of the eye, was isolated independently by McNeil et al. (1997) as well as our group (this report). This gene encodes a homeodomain protein that is most similar to the ARA and CAUP proteins.

In addition to the eye, *mirr* is expressed in the wing disc in a similar, but not identical, pattern as the *iro* genes. In this report, we focus our study on the role of the *mirr* gene in the formation of alula and sensory organs in the wing. Our results suggest that *mirr* is a member of the *iro* complex and acts together with other *iroquois* genes in the prepatterning of sensory organs and alula development.

MATERIALS AND METHODS

Drosophila stocks

B1-12, a *P-lacW* (Bier et al., 1989) insertion mutation, was isolated from an enhancer trap screen (Choi et al., 1996). *B1-12* is allelic to the previously identified mutations, $Sail^1$ (Sai^1) and $Sail^2$ (Sai^2) (Carpenter, 1994) (see Results). *B1-12* homozygotes are semilethal with rare escapers (<1 %). $B1\text{-}12^{g14}$ was isolated from a gamma-ray mutagenesis as described below. Sai^1 and Sai^2, which are both X-ray induced, were provided by A. T. C. Carpenter (Cambridge, England). Both alleles cause dominant outheld wings and recessive lethality while failing to complement each other for the recessive lethality. Sai^1 is associated with *In(3LR)69D2-6; 84E12-F3*. Sai^2 is cytologically normal, but the dominant phenotype maps to 3-37.9 relative to *hairy (h)* and *thread (th)*, consistent with its cytological location at 69D (A. T. C. Carpenter, personal communication). Since *B1-12* and *Sai* mutations are allelic to *mirr* (see Results), we will use the following nomenclature recommended by FlyBase: *B1-12* to $mirr^{B1\text{-}12}$, $B1\text{-}12^{g14}$ to $mirr^{g14}$, Sai^1 to $mirr^{Sai1}$, Sai^2 to $mirr^{Sai2}$. $mirror^{e48}$ ($mirr^{e48}$) was obtained from H. McNeil and M. Simon (Stanford University). McNeil et al. (1997) used '*mrr*' as the symbol for *mirror*. The FlyBase uses '*mirr*' since *mrr* is the symbol for anther gene, *myosin rod-related*. $Tp(3,3)iro^1$, $Df(3L)iro^2$ and iro^{DFM1} were provided by Gomez-Skarmeta and Modolell (Madrid, Spain). The recessive lethality of D^1 is due to a chromosomal inversion break at 69D (Russell et al., 1996) and is labeled in the FlyBase as $mirr^{Dichaete\text{-}1}$. Other mutations are listed in Lindsley and Zimm (1992).

Mutagenesis

To obtain additional alleles of $mirr^{B1\text{-}12}$, the *P-lacW* element was mobilized as described (Choi and Benzer, 1994). We also mutagenized $mirr^{B1\text{-}12}$ chromosomes with gamma irradiation (4000 rad) and screened for flies that have lost w^{+mC}. $mirr^{g14}$, one of the strains isolated from this screen, is recessive lethal and fails to complement all *mirr* mutations.

To isolate mutations that modify the dorsal-specific w^{+mC} expression pattern in the eye of $mirr^{B1\text{-}12}$, $mirr^{B1\text{-}12}$ chromosomes were mutagenized with ethylmethane sulfonate (EMS). *y w;* $mirr^{B1\text{-}12}$*/TM3 Sb* males were fed with 25 mM EMS as described (Lewis and Bacher, 1968) and mated to *y w; D/TM3 Sb*. *y w; (*)* $mirr^{B1\text{-}12}$*/TM3 Sb* flies containing dominant mutations (marked '*') that alter the $mirr^{B1\text{-}12}$ pattern were isolated. Mob^1 (*Modifier of B1-12*) was isolated in this mutagenesis.

Isolation of genomic and cDNA clones

The *P-lacW* element in $mirr^{B1\text{-}12}$ was localized to 69D by in situ hybridization to polytene chromosomes. Genomic DNA adjacent to the *P-lacW* insertion was isolated by plasmid rescue (Pirrotta, 1986). A P1 clone in the *mirr* region (obtained from K. Zinn) and phage clones isolated from a library (a gift of J. Tamkun) were used for physical mapping of the region. A 5 kb *Hin*dIII-*Eco*RI genomic DNA fragment was used as a probe to isolate cDNA clones from an embryonic library (provided by K. Zinn). Preparation of DNA/RNA, blotting and hybridization were carried out using standard method (Sambrook et al., 1989).

A 2.8 kb cDNA was sequentially deleted using the Erase-A-Base (Promega) kit and sequenced by dideoxy chain termination reaction using Sequenase (United States Biochemical Corp.). MIRR protein sequence was compared to the databases using BLAST and FASTA programs. The evolutionary tree for homeoproteins was constructed

using Pedro's BioMolecular Research Tools (Computational Biochemical Research Group).

mRNA expression and immunocytochemistry

For northern blot analysis, total RNA was extracted from flies in different developmental stages using the procedure described by Cathala et al. (1983). Poly(A)$^+$ mRNA was purified using oligo(dT) columns (Pharmacia), separated on a 1% formaldehyde agarose gel and transferred to a Nylon membrane (Amersham). Radioactive *mirr* cDNA probe was generated using the random prime labeling kit (Boehringer Mannheim).

mRNA in situ hybridization to imaginal discs was carried out as described (Choi and Benzer, 1994) using digoxigenin-labeled probes (Tautz and Pfeifle, 1989). Sense and anti-sense DNA probes were generated from a cDNA subclone by polymerase chain reaction.

Immunohistological detection of *lacZ* expression in imaginal discs was carried out using mouse anti-β-gal antibody (Promega) (1:250 dilution) as described (Choi and Benzer, 1994). For double labeling, imaginal discs were incubated with rabbit anti-β-gal antibody (1:100 dilution) and mouse anti-Achaete (AC) antibody (1:1 dilution, from J. Skeath). Secondary antibodies were rabbit Cy3-(1:500 dilution) or mouse FITC-conjugated IgG (1:100 dilution). Immunofluorescence was analyzed using confocal microscopy.

RESULTS

Molecular cloning of *mirror/sail*

The enhancer trap strain *B1-12* was initially identified by the dorsal-specific *white* (w^{+mC}) expression in the eye (Choi et al., 1996). The *P-lacW* element in *B1-12* was localized to 69D by in situ hybridization to polytene chromosomes. Dominant mutations in the *sail* (*sai*) locus were also mapped to the 69D region (Carpenter, 1994; Materials and methods). Unlike normal wings, which are oriented posteriorly in parallel to the body axis, wings of $+/Sai^1$ and $+/Sai^2$ mutants protrude approximately 45° from the body axis. We have found that mutations in *B1-12* and *sai* affect the sensory bristles in the notum and are allelic (see next sections). Because of these observations, a gene nearby the *P-lacW* insertion in *B1-12* was isolated in order to study its expression and role in the development of sensory organs in the notum. Genomic DNA adjacent to the *P-lacW* insertion was isolated by plasmid rescue (Pirrotta, 1986). A P1 and phage genomic clones in the *mirr* region were used for physical mapping of the region. In order to isolate cDNA clones, a 5 kb *Hin*dIII-*Eco*RI genomic DNA fragment (Fig. 1A) was used to probe an embryonic library.

A 2.8 kb cDNA was sequentially deleted and sequenced by dideoxy chain termination reaction. *B1-12* cDNA contained a single long open reading frame encoding a polypeptide of 641 amino acid residues (The nucleotide sequence of *B1-12* cDNA was deposited in the GenBank with the accession number AF004710). This protein sequence was identical to that reported recently by McNeil et al. (1997) (GenBank accession number U95021). Therefore, *B1-12* is renamed hereafter as $mirr^{B1-12}$.

Two genes encoding similar homeoproteins, *ara* and *caup*, are clustered in the 69D chromosomal region and are located within 50 kb from the *mirr* locus (Gomez-Skarmeta et al., 1996; Fig. 1A). Comparison of the predicted MIRR protein sequence with known proteins in the databases revealed the presence of a homeobox domain signature (Fig. 1B) (Gehring et al., 1994a). Seven amino acids, including three residues involved in DNA binding, are conserved in 95% of homeoproteins (Gehring et al., 1994a). Six of these seven residues are conserved in MIRR (Fig. 1B). The predicted secondary structure of the MIRR protein shows helix-turn-helix motif characteristic of most homeodomains (Gehring et al., 1994b) (Fig. 1B). The most closely related proteins are two homeobox proteins of the *iroquois* complex (IROC), Araucan (ARA) (94% identity) and Caupolican (CAUP) (92% identity).

The next homologous group of homeoproteins includes Extradenticle (Exd) (Flegel et al., 1993), a *Drosophila* homolog of the human proto-oncogene product PBX1. The PBX homeodomains are atypical since they contain three extra amino acid residues that are located in the turn between helix1 and helix2 (Fig. 1B). These three residues are involved in cooperative interactions with other homeoproteins to modulate DNA-binding specificity as well as affinity (Chan et al., 1994; van Dijk and Murre, 1994). Although MIRR also contains these extra residues (Fig. 1B), sequence identity between the homeodomains of MIRR and the PBX-family proteins is only about 35%. Analysis of evolutionary distances between the homeodomains shows that the IRO class of homeodomains can be grouped into a distinct subfamily (Fig. 1C).

The *iroquois* complex genes also share similar regions in addition to the homeodomains. A region of highly acidic amino acid residues, which are involved in transcriptional activation (Klemsz and Maki, 1996; Roberts and Green, 1994), can also be found (not shown, see McNeil et al., 1997). MIRR and IRO also contain an EGF (Epidermal Growth Factor)-like motif of Notch, which has been implicated in protein-protein interaction (Lieber et al., 1992). Both ARA and CAUP proteins contain *opa* repeats, stretches rich in glutamine, toward the C-terminal end. However, the presence of an *opa* repeat (Wharton et al., 1985) in MIRR is not as obvious as in ARA and CAUP, although MIRR has some scattered glutamine residues in the C-terminal region (not shown).

A search for similar genes using the EST (Expressed Sequence Tag) databases revealed the presence of highly conserved sequences (over 80% identical) in several species, including the human and mouse, suggesting there may be a family of IRO-like homeoproteins in vertebrates as well.

Expression of *mirr*

Northern blot analysis revealed a single *mirr* transcript of approximately 3.9 kb. The transcript was expressed in all developmental stages (data not shown).

We examined *mirr* expression in the imaginal discs by in situ mRNA hybridization and immunohistological detection of the *lacZ* reporter expression. In the wing disc, *mirr* mRNA is expressed in several regions, including the notum and pleura. *mirr* expression was also detected in the alula region (Fig. 2), an accessory basal structure of the wing (Fig. 3). This expression pattern is very similar to that of *ara* and *caup* (Gomez-Skarmeta et al., 1996). However, *mirr* is not expressed in the precursors for L3/L5 wing veins, tegula and dorsal radius while *ara* and *caup* are expressed in these regions. The tegula is the most proximal part of the anterior wing margin. *lacZ* expression in the wing disc was similar to the mRNA expression pattern (Fig. 2), suggesting that the *lacZ* reporter reflects the pattern of *mirr* expression.

One of the interesting characteristics of $mirr^{B1-12}$ is that the

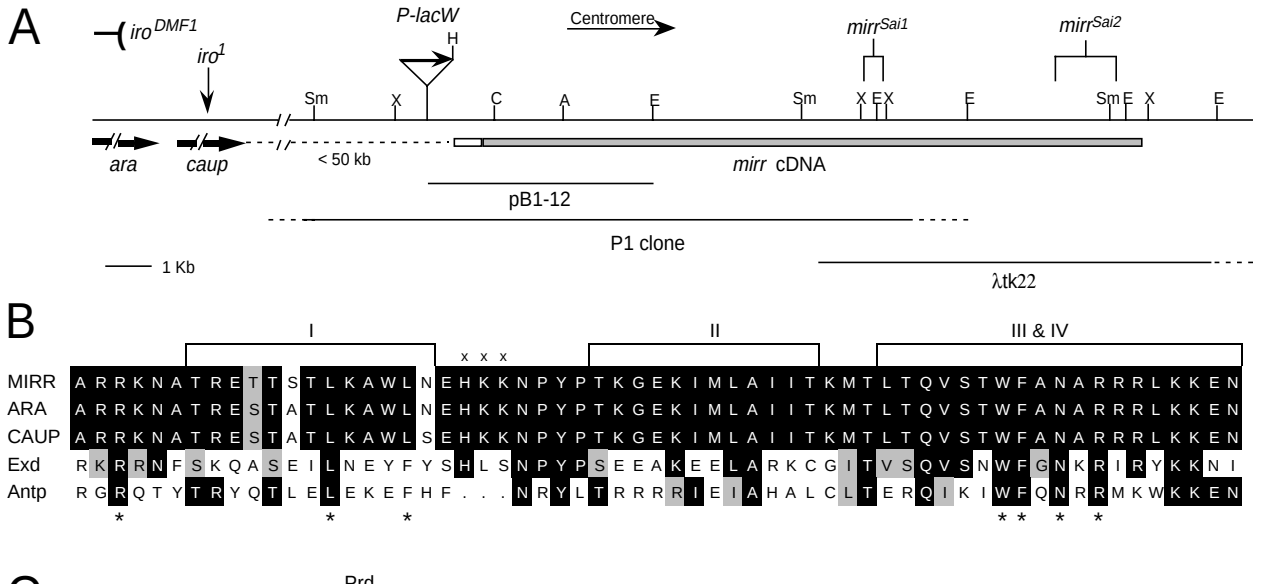

Fig. 1. Physical map and sequence of *mirr*. (A) Physical map of the *mirr* locus. $mirr^{B1\text{-}12}$ has a *P-lacW* element inserted in the *Xba*I-*Cla*I restriction fragment. *ara* and *caup* are indicated by thick arrows, which also indicate the direction of transcription. The region of *ara* and *caup* are not drawn to scale. The distance between *caup* and *mirr* is less than 50 kb (Gomez-Skarmeta et al., 1996). The pB1-12 plasmid was isolated by plasmid rescue (Pirrotta, 1986). The P1 genomic clone contains *mirr* but not *caup*. The dashed lines indicate uncertainties. The 5′-end of the cDNA is approximately 400 bp apart from the *P-lacW* insertion site. The *mirr* cDNA maps in the region shown as the blank and grey bar which indicates the 5′ untranslated region and coding sequence, respectively. The exon-intron boundaries are not determined. Two X-ray alleles, $mirr^{Sai1}$ and $mirr^{Sai2}$, affect the 3′ region of *mirr* as determined by genomic DNA analysis (data not shown). The restriction polymorphism in $mirr^{Sai1}$ is consistent with the distal breakpoint lying between the two *Xba*I sites shown. The proximal breakpoint is at 84E12-F3 (Carpenter, 1994). $mirr^{Sai2}$ is approximately a 1.3 kb deletion, which removes the *Sma*I site. *mirr* is proximal to *caup* (Gomez-Skarmeta et al., 1996). The *iro* mutations used in this study are also indicated (Gomez-Skarmeta et al., 1996). $Tp(3,3)iro^{1}$ has a breakpoint between the first and second exon of *caup*. iro^{DFM1}, which deletes both *ara* and *caup*, has a distal break between the second and third exon of *ara*. $Df(3L)iro^{2}$, a large deletion in the region 69BD, is not shown. The abbreviations for restriction sites are: A, *Apa*I; C, *Cla*I; E, *Eco*RI; H, *Hin*dIII; Sm, *Sma*I; X, *Xba*I. (B) Sequence comparison of homeodomains. The homeodomain is most conserved between MIRR and IRO proteins (93% identity). Black and grey boxes indicate identical and similar amino acid residues, respectively. The next most similar proteins include *Drosophila* Extradenticle (Exd), human PBX and *Drosophila* Antennapedia (Antp). Homeodomains of these proteins are 33-37% identical to that of MIRR. The 4 regions of α-helix characteristic of homeodomains are shown as Roman numbers. Three atypical extra residues (marked with x's) are located between helix 1 and helix 2. Seven amino acids marked by asterisks are conserved in 95% of homeoproteins (Gehring et al., 1994a). (C) Evolutionary tree for homeodomain proteins. Representative members from 25 subfamilies of homeoproteins described in Gehring et al. (1994a) were compared to construct an evolutionary tree. The IRO family proteins, including MIRR, constitute a new branch of homeodomains. The $mirr^{B1\text{-}12}$ sequence has been deposited in the GenBank under the accession number AF004710.

mini white^{+} gene (w^{+mC}) in the *P-lacW* enhancer trap element is expressed specifically in the dorsal region of the eye. *mirr* mRNA expression is also restricted to the dorsal region of the eye disc (Fig. 2A). The dorsal-specific expression was also detected in undifferentiated second instar eye discs, suggesting that dorsoventral specification occurs prior to retinal morphogenesis (data not shown). We also examined the pattern of *lacZ* expression in the $mirr^{B1\text{-}12}$ enhancer trap strain. Eye discs from $mirr^{B1\text{-}12}$ third instar larvae also showed *lacZ* expression restricted to the dorsal region. *lacZ* was more abundantly expressed in the polar region of the dorsal half and anterior to the morphogenetic furrow (Fig. 2B).

mirr is required for alula formation and wing positioning

$mirr^{B1\text{-}12}$ is semi-lethal over both $mirr^{Sai1}$ and $mirr^{Sai2}$ alleles. In rare cases, $mirr^{B1\text{-}12}/mirr^{Sai}$ heterozygous adult flies were found. All $mirr^{B1\text{-}12}/mirr^{Sai}$ adults showed outheld wings similar to $+/mirr^{Sai}$ wings. In addition, alulae are completely absent in $mirr^{B1\text{-}12}/mirr^{Sai}$ (Fig. 3C). The lack of alulae is also a characteristic of *Dichaete* mutants (Lindsley and Zimm, 1992) (Fig. 3D), although *mirr* and *D* are different genes. Dominant phenotypes of $mirr^{Sai}$ and D^{1} are not identical, since alulae are missing in $+/D^{1}$ wings but not in $+/mirr^{Sai}$ wings (Fig. 3B, D). Wings found in $mirr^{B1\text{-}12}/mirr^{g14}$ and $mirr^{B1\text{-}12}/mirr^{e48}$ flies are

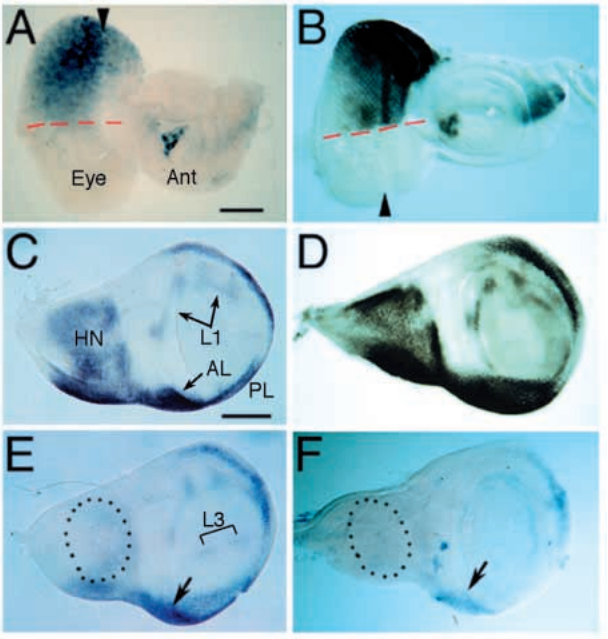

Fig. 2. *mirr* expression in imaginal discs. Eye (A,B) and wing discs (C-F) were dissected from third instar larvae and hybridized with digoxigenin-labeled *mirr* cDNA probe (A,C,E,F) or immunostained with anti-β-gal antibody (B,D). In the eye discs, anterior is to the right and dorsal is up. Distal is to the right and anterior is up for the wing discs. (A) mRNA expression in the wild-type eye/antenna (Ant) disc. *mirr* expression is restricted to the dorsal region of the eye disc. The red dashed line indicates the dorsoventral midline. The position of the morphogenetic furrow is marked by arrowhead. (B) *lacZ* expression in the $+/mirr^{B1\text{-}12}$ eye/antenna disc. *lacZ* staining is restricted to the dorsal region above the equator (dashed line). (C) Normal mRNA expression in the $+/mirr^{B1\text{-}12}$ wing disc. The *mirr* transcript can be detected in the hemi-notum (HN), ventral pleura (PL), alula (AL), and L1 vein (L1). (D) *lacZ* expression in the $+/mirr^{B1\text{-}12}$ wing disc. *lacZ* is expressed in a similar pattern as *mirr* mRNA. (E) In $mirr^{B1\text{-}12}/mirr^{B1\text{-}12}$ disc, *mirr* mRNA expression is reduced in the heminotum (dotted circle) but is relatively normal in the alula region (arrow). It also shows weak ectopic expression in the L3 vein. (F) $mirr^{B1\text{-}12}/mirr^{Sai1}$ disc shows little *mirr* expression, especially in the heminotum. The expression in the alula region is present in $mirr^{B1\text{-}12}/mirr^{B1\text{-}12}$ but greatly reduced in $mirr^{B1\text{-}12}/mirr^{Sai1}$ (arrow). The scale bars are 50 μm (A,B), or 75 μm (C-F).

also heldout and deficient in alulae, identical to those found in $mirr^{B1\text{-}12}/mirr^{Sai1}$ flies. Therefore, the loss of alula is a recessive phenotype of *mirr* mutations. Since some $mirr^{B1\text{-}12}/mirr^{g14}$ and $mirr^{B1\text{-}12}/mirr^{e48}$ can survive to the adult stage, while $mirr^{g14}/mirr^{e48}$ is 100% lethal, $mirr^{B1\text{-}12}$ appears to be a weaker allele than $mirr^{g14}$ and $mirr^{e48}$.

mirr expression is greatly reduced in the notum region of the wing disc of $mirr^{B1\text{-}12}$ homozygous flies, but there is significant expression in the alula region (Fig. 2E), consistent with the relatively normal development of the alula in these flies. In contrast, $mirr^{B1\text{-}12}/mirr^{Sai1}$ showed a severe reduction of *mirr* expression in the alula region as well as the notum, suggesting that this heterozygote represents a strong hypomorph or null in these tissues. These results are consistent with the lack of alula in $mirr^{B1\text{-}12}/mirr^{Sai1}$ flies (Figs 2F, 3C) and the requirement of $mirr^{+}$ for the formation of alula and normal positioning of the wing.

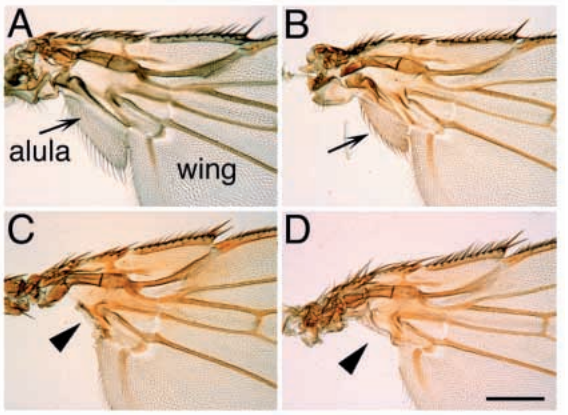

Fig. 3. Lack of alula in *mirr*. Effects of *mirr* mutations on alula formation. (A) Wild-type alula (arrow). (B) In $+/mirr^{Sai1}$, wings are outheld (not shown). The alula develops normally although it is slightly smaller than that of the wild-type. (C) $mirr^{Sai1}/mirr^{B1\text{-}12}$ is semi-lethal but escapers show outheld wings and the complete lack of alula (arrowhead). (D) $+/D^{1}$ also shows no alula (arrowhead). The scale bar is 200 μm.

mirr is required for the formation of sensory organs in the lateral heminotum

An individual bristle on the notum can be easily identified by its specific position. *mirr* mutations caused specific loss of macrobristles only in the lateral domain of the notum. $mirr^{Sai1}/TM3\ Sb$ or $mirr^{Sai1}/TM6$ heterozygotes showed dominant bristle phenotype: deletion of presutural (PS) and/or posterior supraalar (pSA) bristles (Fig. 4B, Table 1). In many cases (31%), both PS and pSA bristles were absent. The chromosomes that were used to balance $mirr^{Sai1}$ mutation (*TM3 Sb* or *TM6 Tb*) did not show any significant effects on the bristle pattern (Fig. 4A, Table 1). $mirr^{B1\text{-}12}$ homozygous flies also showed consistent bristle defects. Approximately 90% of $mirr^{B1\text{-}12}/mirr^{B1\text{-}12}$ flies were missing one bristle, although occasionally two bristles were deleted. The deletion of bristles was specifically restricted to two of the seven macrobristles in the region; the pSA and anterior postalar bristles (aPA) (Fig. 4C, Table 1). Similar bristle defects were also found in $mirr^{B1\text{-}12}/mirr^{g14}$ and $mirr^{B1\text{-}12}/mirr^{E48}$ (data not shown). Another dominant allele, $+/mirr^{Sai2}$ displayed normal macrobristle formation, indicating that the effects on the bristles are allele-specific. However, the PA bristles were selectively removed in $mirr^{B1\text{-}12}/mirr^{Sai2}$, as seen in $mirr^{B1\text{-}12}/mirr^{B1\text{-}12}$ (Table 1). The strongest phenotype observed was found in $mirr^{Sai1}/mirr^{B1\text{-}12}$, in which up to four lateral bristles were missing (Table 1). This is consistent with our observation that *mirr* mRNA level is greatly reduced in the notum of this mutant wing disc (Fig. 2F). Our results indicate that the elimination of lateral macrobristles by *mirr* mutations are allele-specific and are mainly restricted to four bristles; the PS, pSA, aPA and pPA. This is consistent with the expression of *mirr* in this subset of sensory organ precursors (SOPs) (see Fig. 5 in the next section). The loss of only 1 to 3 macrobristles in weaker allelic combinations might be due to residual $mirr^{+}$ activity or partial compensation by iro^{+} expression in the heminotum region. It is important to note that the PA bristles and alula are affected in $mirr^{Sai}/mirr^{B1\text{-}12}$ but not in $+/mirr^{Sai}$ or $+/mirr^{B1\text{-}12}$. Therefore, the removal of these

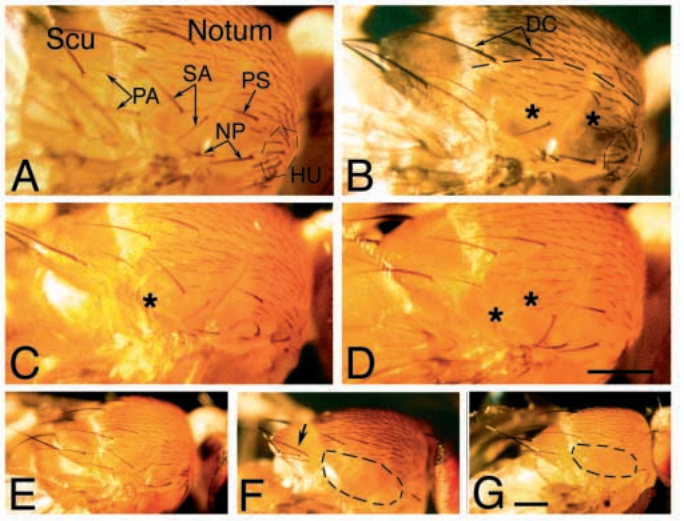

Fig. 4. Loss of bristles in lateral notum by *iro* and *mirr* mutations. (A) *+/TM6 Tb*. Normal pattern of seven macrobristles is shown in the lateral subdivision of the notum (the region lateral to the dashed line in B). The *TM6 Tb* chromosome has a dominant mutation, *Humeral (Hu)*, which induces more hairs in the humeral (dashed circles) but has no effect on the bristles located on the notum and scutellum (See (E) *Canton-S* wild type for comparison). *TM6 Tb* was used as a control for $mirr^{Sai1}$*/TM6 Tb*. (B) $mirr^{Sai1}$*/TM6 Tb*. The presutural (PS) and the posterior supraalar (SA) bristles are missing. The missing bristles are marked with asterisks. Note that the dorsocentral bristles (DC, arrows) are not affected in any *mirr* mutants. (C) $mirr^{B1\text{-}12}/mirr^{B1\text{-}12}$. The anterior postalar (PA) bristle is missing. (D) $iro^{DFM1}/mirr^{B1\text{-}12}$. The posterior supraalar as well as the anterior postalar bristles are absent. (E) Canton-S wild type. (F) Lateral macrobristles are entirely absent in iro^{1}/iro^{2} (dotted circle). The iro^{1}/iro^{2} flies have shorter dorsocentral bristles than the normal since they carry *stubbloid*^{2} (*sbd*^{2}) mutations on both third chromosomes. *sbd*^{2} itself does not affect the number of bristles. Note also that this *iro* mutant has more anterior scutellar bristles (3 instead of 1) than the normal (arrow). Duplication of anterior scutellar bristles is a common phenotype in *mirr* mutants as well. (G) The entire group of lateral bristles is also removed in iro^{1}/Mob^{1} $mirr^{B1\text{-}12}$, similar to iro^{1}/iro^{2}. HU, Humeral; PS, presutural bristle; NP, notopleural bristles; PA, postalar bristles; SA, Supraalar bristles; Scu, Scutellum. Anterior is to the right. The scale bars are 300 μm.

Table 1. Effects of *mirr* mutations on the lateral bristles

Macrobristles	$mirr^{Sai1}$ / *TM6 Tb*	$mirr^{B1\text{-}12}$ / $mirr^{B1\text{-}12}$	$mirr^{Sai1}$ / $mirr^{B1\text{-}12}$	$mirr^{Sai2}$ / $mirr^{B1\text{-}12}$	Mob^{1} $mirr^{B1\text{-}12}$ / *TM6 Tb*
presutural	49	100	0	100	100
A. notopleural	100	99	100	100	100
P. notopleural	100	100	100	100	100
A. supraalar	92	99	100	100	100
P. supraalar	59	58	0	100	62
A. postalar	100	44	0	25	56*
P. postalar	100	100	25	0	100

The lateral heminotum was scored for the presence of macrobristles. The numbers represent the percentage of bristles that are present. The (*) indicates duplication of a bristle. Note that the presutural, posterior supraalar, anterior postalar and posterior postalar bristles (highlighted by shading) are preferentially affected by *mirr* mutations (see also Fig. 5D). The number of heminotum scored was 90, 109, and 76 for $mirr^{Sai1}$*/TM6 Tb*, $mirr^{B1\text{-}12}/mirr^{B1\text{-}12}$, and Mob^{1} $mirr^{B1\text{-}12}$*/TM6 Tb*, respectively. $mirr^{Sai1}/mirr^{B1\text{-}12}$ and $mirr^{Sai2}/mirr^{B1\text{-}12}$ are almost completely lethal (99%). 10 escaper flies were scored for bristle defects. Approximately 95% of $mirr^{B1\text{-}12}$ heterozygotes (over *TM3 Sb* or *TM6 Tb* balancer) showed normal bristle pattern (not listed in the Table) while $+/mirr^{Sai1}$ and $mirr^{B1\text{-}12}$ homozygotes significantly affect the number of lateral macrobristles. In $mirr^{Sai1}$*/TM6 Tb*, either the presutural or posterior supraalar bristle was deleted. In $mirr^{B1\text{-}12}$ homozygote, either the posterior supraalar or anterior postalar was removed. The Mob^{1} mutation dominantly reduces the level of *mirr* expression (also see Fig. 6). In Mob^{1} $mirr^{B1\text{-}12}$*/TM6 Tb*, the posterior supraalar was absent, but the anterior postalar was duplicated. 'A' and 'P' in front of bristle names indicate anterior and posterior, respectively.

bristles and alula is a recessive phenotype of $mirr^{Sai}$ and $mirr^{B1\text{-}12}$. On the contrary, the PS bristle is missing in $+/mirr^{Sai1}$ but not in $mirr^{Sai1}/mirr^{B1\text{-}12}$, indicating that the lack of PS is a dominant phenotype of $mirr^{Sai1}$.

Genetic interactions between *mirr* and *iroquois* mutations

Clonal analysis has shown that *iro* gene function is required for alula formation (Gomez-Skarmeta et al., 1996). Since *mirr* and *iro* genes are similar in structure and function, we tested whether *mirr* mutations show genetic interactions with *iro* mutations.

iro^{DFM1} is a deletion that causes embryonic or early larval lethality (Gomez-Skarmeta et al., 1996). $iro^{DFM1}/mirr^{B1\text{-}12}$ is semi-lethal with rare escapers (less than 4% of progeny from a cross between $mirr^{B1\text{-}12}$*/TM3* and iro^{DFM1}*/TM3*, see Table 2). $iro^{DFM1}/mirr^{B1\text{-}12}$ flies that escaped lethality showed outheld wings and lack alulae, which is identical to wings seen in $mirr^{Sai}/mirr^{B1\text{-}12}$(Fig. 3C, Table 2) and $mirr^{g14}/mirr^{B1\text{-}12}$ (data not shown). The failure of the iro^{DFM1} deletion to complement $mirr^{B1\text{-}12}$ might be explained if the deletion uncovers the neighboring *mirr* locus. However, this is not the case since a normal level of *mirr* transcript can be detected in iro^{DFM1} homozygous embryos (data not shown). Therefore, the lack of alula and the abnormal wing positioning in $iro^{DFM1}/mirr^{B1\text{-}12}$ flies appears to be due to genetic interaction between the *iro* and *mirr* genes. 50% reduction of *mirr* and *iro* activity together might be sufficient to cause the wing and alula phenotypes. Alternatively, it is also possible that the iro^{DFM1} deletion affects wing-specific enhancers shared by both *mirr* and *iro*, thereby causing wing and alula defects seen in $iro^{DFM1}/mirr^{B1\text{-}12}$.

The *iro* genes are also required for the formation of macrobristle sensory organs in the lateral notum (Dambly-Chaudiere and Leyns, 1992; Gomez-Skarmeta et al., 1996). Both $+/mirr^{B1\text{-}12}$and $+/iro^{DFM1}$ heterozygotes do not show significant defects in the macrobristles. However, $iro^{DFM1}/mirr^{B1\text{-}12}$ *trans*-heterozygotes showed the deletion of the pSA and/or aPA bristles (Fig. 4D, Table 2), similar to the defects seen in $mirr^{B1\text{-}12}/mirr^{B1\text{-}12}$ (Fig. 4C, Table 1). This suggests that the *mirr* and *iro* genes interact in the formation of sensory organs as well as alula.

iro^{1}, a recessive mutation associated with a transposition *Tp(3,3)iro*^{1}, is probably a null allele for *caup* but not for *ara* (Skarmata-Gomez et al., 1996). iro^{1}/iro^{1} and iro^{1}/iro^{2} (iro^{2} is a deletion uncovering the *iro* locus) eliminates all macrobristles in the lateral notum (Fig. 4F). $iro^{1}/mirr^{B1\text{-}12}$ flies

Table 2. Genetic interaction between *mirr* and *iro* mutations

	$mirr^{B1-12}$			Mob^1 $mirr^{B1-12}$		
	Lethality	Alula	Bristles missing (%)	Lethality	Alula	Bristles missing (%)
iro^1	viable	normal (100%)	PS (14%) aSA* (21%) pSA^{++} (50%) aPA (7%)	viable	normal (100%)	all lateral bristles (100%)
iro^{DFM1}	semi-lethal (98% die)	absent (100%)	pSA, aPA (% ND)	lethal	NA	NA
iro^2	semi-lethal (98% die)	absent (100%)	pSA (100%) aPA (10%) pPA (100%)	lethal	NA	NA

yw; $mirr^{B12}$/TM3 Sb or *yw; Mob^1 $mirr^{B1-12}$/TM3 Sb* was crossed with *iro* alleles to test for complementation. iro^{DFM1} and iro^2 are deletions in the *iro* region while iro^1 is a transposition with a breakpoint at the *caup* gene (Materials and methods). While $mirr^{B1-12}/iro^1$ flies are normal, $mirr^{B1-12}/iro^{DFM1}$ show outheld wings and lack alulae. $mirr^{B1-12}/iro^{DFM1}$ and $mirr^{B1-12}/iro^{iro2}$ are both lethal but about 2% of flies escape lethality. Mob^1 $mirr^{B1-12}$ is completely lethal with iro^{DFM1} or iro^2. Mob^1 $mirr^{B1-12}$ is viable with iro^1, but shows complete lack of macrobristles in the lateral notum (Fig. 4G). Severe *mirr* alleles, $mirr^{g14}$ and mrr^{e48}, behave similarly as the Mob^1 $mirr^{B1-12}$. (++) indicates duplication of the pSA bristle. (*) The absence of the aSA bristle in $iro^1/mirr^{B1-12}$ is due to the dominant effect of iro^1 since $+/iro^1$ also shows the deletion of aSA. PS, Presutural; pSA, posterior Supraalar; aPA, anterior postalar; pPA, posterior postalar. ND, Not determined; NA, not available.

were viable with no obvious defects in the alula. However, they showed a deletion of PS and aPA, as seen in $mirr^{B1-12}/mirr^{B1-12}$ (Fig. 4C), or a duplication of pSA (Table 2). We have isolated a mutation Mob^1 (for *Modifier of B1-12*) that enhances $mirr^{B1-12}$ phenotypes (see Materials and methods and next section for the origin of Mob^1 mutation). The Mob^1 mutation was tested for enhancement of the $mirr^{B1-12}$ phenotype in the lateral bristles. Strikingly, iro^1/Mob^1 $mirr^{B1-12}$ resulted in the complete removal of the lateral macrobristles (Fig. 4G). These results also show a correlation of the severity of *mirr* mutant alleles and the strength of phenotypic interaction with *iro* mutations. Mob^1 also showed a specific dominant effect on a lateral bristle. The pSA and aPA bristles were deleted and duplicated, respectively, in Mob^1 $mirr^{B1-12}/TM6$ *Tb* (Table 1).

MIRR and AC are coexpressed in a subset of sensory organ precursors

ARA and CAUP are expressed in SOPs, acting as positive transcriptional regulators of *ac-sc* in the wing disc epithelium (Gomez-Skarmeta et al., 1996). We examined whether MIRR is also expressed in the SOPs in the notum. Wing discs from $mirr^{B1-12}$ double stained with anti-β-gal and anti-Achaete antibody showed that MIRR and AC expression overlap only

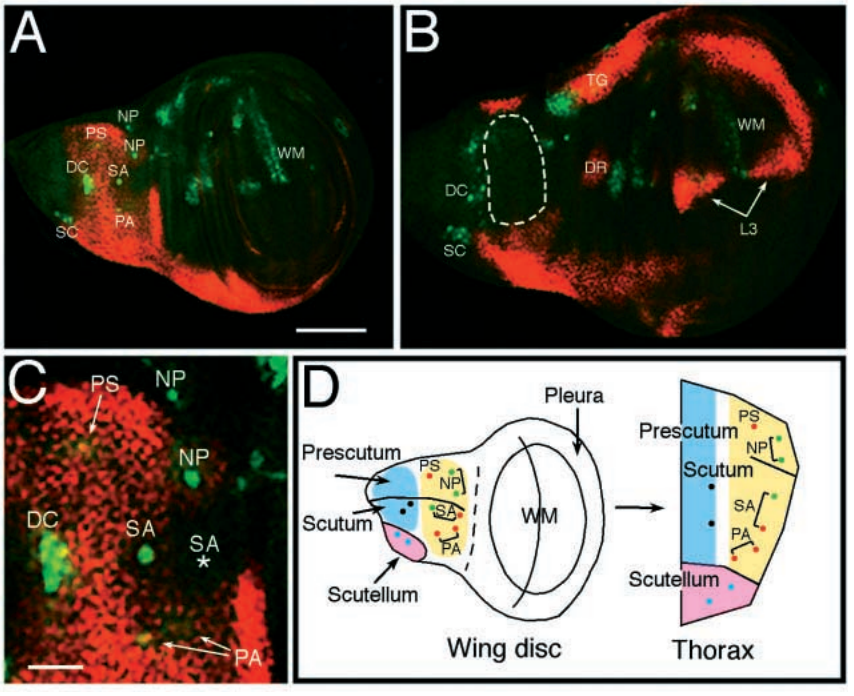

Fig. 5. Distribution of MIRR and AC protein in the wing disc. Wing discs from $mirr^{B1-12}/TM3$ *Sb* and iro^1/Mob^1 $mirr^{B1-12}$ were stained with anti-β-gal and anti-AC antibodies as described (Materials and methods) with Cy3- (red for β-galactosidase) or FITC-conjugated secondary antibody (green for AC). Immunofluorescence from double stained discs were visualized using confocal microscopy. (A) $mirr^{B1-12}/TM3$ *Sb*. Not all SOPs were detected with anti-AC antibody in most disc specimens. This panel shows six of seven AC-positive SOPs detected in the lateral heminotum (yellow region in D). The SOP for the posterior supraalar is not seen (asterisk in C). (B) iro^1/Mob^1 $mirr^{B1-12}$. MIRR expression is greatly reduced in the heminotum. No SOPs for lateral bristles are detected while SOPs for dorsocentral and scutellar bristles are present. Note that MIRR is ectopically expressed in the L3 wing vein, tegula, and dorsal radius. (C) High magnification view of Fig. 5A. Both MIRR and AC are co-expressed in the SOPs for presutural and postalar bristles (orange color). Some dorsocentral bristle precursors also appear to express MIRR and AC. (D) A scheme of wing imaginal disc and adult notum showing positions of SOPs in the notum. The lateral notum, central notum and scutellum are colored with yellow, blue and purple, respectively. SOPs for dorsocentral and scutellar bristles are indicated by black and blue dots, respectively. Lateral SOPs are marked with green dots. The red dots indicate sensory organs which are affected by *mirr* mutations. DC, Dorsocentral; SC, scutellum; PS, presutural; NP, notopleural; SA, supraalar; PA, postalar; WM, wing margin; DR, dorsal radius; TG, tegula. Anterior is up. The scale bars are 75 μm (A,B), or 15 μm (C).

in a subset of bristle SOPs in the lateral heminotum, including the PS and PA bristles, which are affected by *mirr* mutations (Fig. 5C). In contrast, SOPs for the notopleural and anterior supraalar bristles are stained with anti-AC but not with anti-β-gal antibody, suggesting that MIRR is either not expressed or is expressed at low levels in the notopleural (NP) and aSA bristles. This is consistent with our findings that these bristles form normally in *mirr* mutations. These results suggest that MIRR as well as ARA and CAUP might control *ac-sc* expression, and the loss of a subset of bristles in different *mirr* alleles might result from the loss of the corresponding SOPs rather than the degeneration of bristles. To confirm the absence of SOPs responsible for the formation of the PS, pSA and/or PA bristles, we examined *mirr* mutants for expression of AC or *A101 lacZ* reporter, a marker for SOPs in imaginal discs (Huang et al., 1991). Although there was a consistent reduction in the number of anti-AC (or anti-β-gal)-positive SOPs in the mutant discs, it was difficult to interpret the results since wild-type wing disc did not show all 7 lateral SOPs in most discs examined. This is probably due to low levels of AC expression or transient expression. Therefore, we examined iro^{1}/Mob^{1} $mirr^{B1\text{-}12}$ heterozygote since the entire lateral bristles are removed in the presence of Mob^{1}, a dominant modifier mutation that enhances $mirr^{B1\text{-}12}$ phenotype (Fig. 4G). In iro^{1}/Mob^{1} $mirr^{B1\text{-}12}$ wing discs, all SOPs in the lateral heminotum were eliminated as expected (Fig. 5B). This is consistent with our speculation that the loss of bristles in *mirr* mutations is due to the loss of corresponding SOPs.

Recovery of *white*$^{+}$ expression by *Modifier of B1-12*

In an attempt to identify genes that might regulate the dorsal-specific expression of w^{+mC} gene, we searched for dominant mutations that alter the pattern of dorsal w^{+mC} expression. Mob^{1}, one of these mutations, disrupts the dorsal specificity of w^{+mC} gene expression in the eye of $mirr^{B1\text{-}12}$ flies. The Mob^{1} mutation slightly reduces the level of dorsal w^{+mC} expression from red, as seen in $mirr^{B1\text{-}12}$, to orange. Mob^{1} also causes a dramatic derepression of the ventrally silenced w^{+mC}, resulting in pigmentation in the ventral region of the eye (Fig. 6D).

The Mob^{1} mutation is homozygous lethal. It is also lethal over all tested *mirr* mutations, including the dominant alleles $mirr^{Sai1}$, $mirr^{Sai2}$ and $mirr^{Dichaete\text{-}1}$, as well as the recessive alleles $mirr^{g14}$ and $mirr^{e48}$. Furthermore, the Mob^{1} mutation enhances weak *mirr* phenotypes. As mentioned earlier, $mirr^{B1\text{-}12}$ homozygotes that escaped from lethality show mild bristle defects but have normal alula. However, Mob^{1} $mirr^{B1\text{-}12}/mirr^{B1\text{-}12}$ results in the complete elimination of the alula (data not shown). Mob^{1} appears to be located very close to $mirr^{B1\text{-}12}$ since we have screened over 11,000 progeny but have failed to separate the two mutations by meiotic recombination (data not shown).

Since Mob^{1} derepresses the ventrally silenced w^{+mC} in the $mirr^{B1\text{-}12}$ eye, we examined whether Mob^{1} also alters *mirr* expression using the *lacZ* reporter in the $mirr^{B1\text{-}12}$ enhancer trap. In Mob^{1} mutants, *lacZ* expression was greatly reduced in the eye as well as in the wing disc (Fig. 6E,F). Interestingly, Mob^{1} induced ectopic *lacZ* expression in the L3 wing vein precursor, tegula and dorsal radius areas, where *mirr* is not normally expressed (Figs 5B, 6F). These results suggest that Mob^{+} plays dual roles in silencing or enhancing *mirr* expression in different regions of tissue. In the eye, Mob^{+} is required for normal level of *mirr* expression in the dorsal half and for the silencing of w^{+mC} in the ventral half. In the wing, Mob^{+} enhances *mirr* expression in most regions but silences it in the L3 vein, tegula and dorsal radius.

DISCUSSION

mirr is required for alula and sensory organ formation

ARA and CAUP can functionally replace each other for activation of the proneural genes *ac-sc* (Gomez-Skarmeta et al., 1996). The coexpression of *mirr* and *iro* genes in some regions of the wing disc suggests that these genes may share *cis*-regulatory sequences. However, *mirr* and the *iro* genes show different expression patterns in the wing vein area. The *iro* genes are expressed and required for normal development of wing veins L1, L3 and L5. However, *mirr* is not apparently involved in wing vein formation since it is not expressed at significant levels in this area. Correspondingly, *mirr* mutants do not show any wing vein defects.

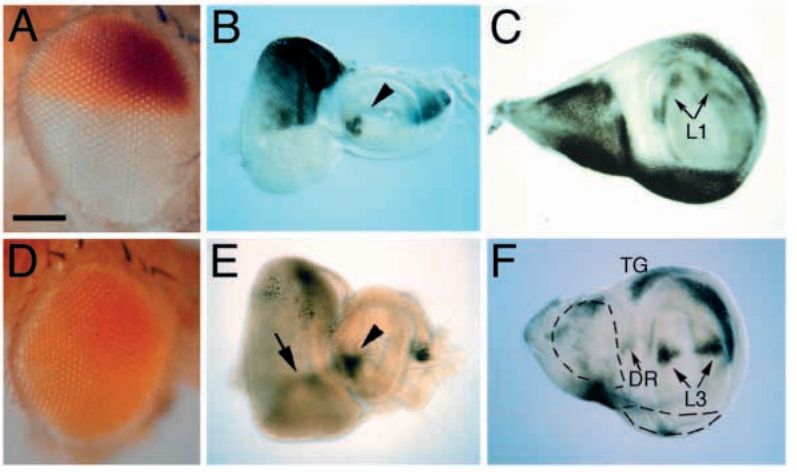

Fig. 6. Effects of the *Mob* mutation on *white* and *mirr* expression. The dorsal-specificity of w^{+mC} expression in $mirr^{B1\text{-}12}$ was modified by the *Mob* mutation. (A) Expression of w^{+mC} in $mirr^{B1\text{-}12}$. (B) *lacZ* reporter expression in the $mirr^{B1\text{-}12}$ eye disc (same as Fig. 2B). (C) *lacZ* reporter expression in the $+/mirr^{B1\text{-}12}$ wing disc (same as Fig. 2D). (D) Expression of w^{+mC} in $+/Mob^{1}$ $mirr^{B1\text{-}12}$. The level of w^{+mC} expression in the dorsal half is reduced and the ventral repression of w^{+mC} is derepressed. (E) *lacZ* reporter expression is greatly reduced in the dorsal domain of the $+/Mob^{1}$ $mirr^{B1\text{-}12}$ eye disc. Mob^{1} causes weak *lacZ* expression in the ventral region (arrow). The expression in the antenna disc (arrowhead) is less affected by Mob^{1}. (F) *lacZ* reporter expression in the $+/Mob^{1}$ $mirr^{B1\text{-}12}$ wing disc. The Mob^{1} mutation eliminates most of *mirr* reporter expression in the L1 vein, heminotum, ventral pleura, and alula (dashed circles). Note that *mirr* is ectopically expressed in the wing vein L3, tegula (TG), dorsal radius (DR) in the Mob^{1} mutant (see also Fig. 5B). The scale bars are 100 μm (A,D), 80 μm (B,E), or 120 μm (C,F).

The alula is a basal structure of the wing located in the hinge region between the notum and the base of the wing veins (Bryant, 1975). *iro* genes are required for alula formation (Gomez-Skarmata et al., 1996). However, since *iro* mutations used in this study were large deletions affecting both *ara* and *caup*, it is unclear whether both *ara* and *caup* are essential for alula formation. On the contrary, *mirr* alone is essential for alula formation (Fig. 3). Our results on genetic interactions between the *mirr* and *iro* genes suggest that they act in the same or a parallel pathway for alula formation.

The D^1 mutation causes similar dominant wing phenotypes as *mirr* mutations, that is, outheld wings with no alula (Fig. 3D). The D^1 chromosome contains an inversion that has breakpoints at 70D1-2 and 69D, where *D* and *mirr* are located, respectively. The lethality of the D^1 homozygote is due to a chromosomal break at 69D, named $mirr^{Dichaete\text{-}1}$ (Materials and methods). The dominant *D* phenotype is caused by misexpression of the D protein (or SOX70D) in the wing, which contains the SOX DNA-binding domain (Russell et al., 1996). *mirr* mRNA expression is significantly reduced in the $+/D^1$ wing disc (unpublished results). It is possible that SOX70D misexpression in the wing might interfere with transcription of *mirr*, resulting in the failure of alula development. The SOX domain, found in proteins of the HMG (High Mobility Group) superfamily, has the capability of DNA bending upon binding (Ferrari et al., 1992; Giese et al., 1992). DNA bending in the regulatory region of *mirr* by misexpressed SOX70D and subsequent reduction of *mirr* expression might be a mechanism for the dominant *D* wing phenotype.

Different *mirr* mutations affect different lateral bristles (Table 1). Such allele specificity was also found in the *ac-sc* mutations (Lindsley and Zimm, 1992). Different alleles of *ac-sc* mutations eliminate specific proneural clusters and the corresponding sensory organs (Cubas et al., 1991; Gomez-Skarmeta et al., 1995). Proneural clusters in the wing disc are constructed by spatiotemporally specific enhancer elements of *ac-sc*. Each enhancer drives *ac-sc* expression in only one or a few proneural clusters (Ruiz-Gomez and Modolell, 1987; Gomez-Skarmeta et al., 1995). Similarly, it is possible that different *mirr* alleles might have mutations in different regulatory regions that control the development of different lateral bristles. It was also noted that *mirr* mutations affect specifically four of the seven macrobristles in the lateral heminotum: presutural (PS), posterior supraalar (pSA) and two postalar (PA) bristles. This is strongly correlated with high levels of MIRR expression in this subset of SOPs (Fig. 5). MIRR appears to be involved in the formation of the PS, pSA and PA bristles while IRO or a combination of IRO and MIRR are required in the other bristles of the lateral notum.

Since MIRR is structurally and functionally similar to ARA and CAUP, it might also be involved in the direct transcriptional activation of *ac-sc*. Therefore, bristle defects found in *mirr* mutants might be caused by reduced transcription of *ac-sc*. This is consistent with our observation that the bristles as well as AC expression in the lateral notum in the wing disc are eliminated in $iro^1/Mob^1 mirr^{B1\text{-}12}$. Alternatively, MIRR might also act as an upstream activator of the *iro* genes. Since *mirr* is expressed normally in embryos that are homozygous for the iro^{DFM1} deletion, *mirr* is not likely to be downstream of the *iro* genes (unpublished results).

Subdivision of the notum

The notum can be divided into at least three longitudinal genetic subdomains (Calleja et al., 1996). The central domain is marked by the expression of *pannier (pnr)* (Ramain et al., 1993). The *pnr* domain is laterally bounded by *em462* gene expression. It was suggested that the most lateral domain may be defined by the *iro* genes (Calleja et al., 1996). This is consistent with our results that mutations in *mirr* specifically reduce the number of macrobristles in the lateral domain but do not affect bristles located more medially (Fig. 5D). A critical boundary between the *pnr* and *em462* domain appears to be the longitudinal line connecting the two dorsocentral bristles (dotted lines in Fig. 4B), which coincides with the stripe of *wingless (wg)* expression (Calleja et al., 1996). The boundary defined by *mirr* and the other *iro* genes is probably located more lateral than the *wg* domain since the dorsocentral bristles are not affected by *mirr* mutations. Unlike the A/P and D/V compartment boundaries that are established early by cell lineage, the longitudinal borders between these notum domains are not lineage boundaries (Calleja et al., 1996). It was suggested, therefore, that secreted WG protein might play an inductive role for the formation of the boundary between the *pnr* and *em462* domains. Candidate molecules involved in the induction of the *iro* domain boundary have not yet been identified.

It has been proposed that specific *ac-sc* enhancers interact with site-specific transcriptional regulators of *ac-sc* (Ghysen and Dambly-Chaudiere, 1988, 1989), which function as prepattern genes. Hairy, a basic-helix-loop-helix protein, is one such regulator that acts as a transcriptional repressor by binding to an *ac* regulatory sequence (Rushlow et al., 1989; Skeath and Carroll, 1991; Ohsako et al., 1994; Van Doren et al., 1994). ARA and CAUP are positive regulators of *ac-sc* and bind directly to the *ac-sc* enhancers (Gomez-Skarmeta et al., 1996). The striking similarities between MIRR, ARA and CAUP suggest that at least three *iro* complex genes play roles in prepatterning the sensory organs of the lateral notum by controlling *ac-sc* expression.

Dorsoventral pattern in the eye

Photoreceptor clusters are arranged in opposite orientations in the dorsal and ventral halves of the compound eye, resulting in mirror symmetry about the equator (Ready et al., 1976). $mirr^{B1\text{-}12}$ as well as similar enhancer trap lines have been isolated that show w^{+mC} expression restricted to the dorsal region (Sun et al., 1995; Brodsky et al., 1996; Choi et al., 1996; McNeil et al., 1997). These lines suggest that there are genetic distinctions between the dorsal and ventral domain of the eye, although the equator is not a cell-lineage boundary (Ready et al., 1976). Since *mirr* is expressed in a dorsal-specific pattern in undifferentiated second instar eye discs (data not shown), the D/V boundary appears to be established prior to the initiation of retinal differentiation. It is an interesting possibility that the dorsoventral boundary defined by *mirr* gene expression might play a role in the formation of the equator. Some defects in dorsoventral polarity were found in *mirr* mutants (McNeil et al., 1997). However, *mirr* may not be the sole determinant of the equator since *mirr* mutations are not always sufficient to cause severe polarity defects (McNeil et al., 1997).

The dorsal-specific expression of *mirr* mRNA and w^{+mC} suggests that there is a ventrally expressed gene that acts on the regulatory region of *mirr* to silence its expression. Out results suggest that Mob^{+} is required for the silencing of w^{+mC} and *mirr* in the ventral domain of the eye since w^{+mC} is derepressed in the *Mob* mutant. Mob^{+} is also necessary for normal level of *mirr* expression in the dorsal domain of the eye. It appears, therefore, that Mob^{+} is necessary for silencing and enhancing in the ventral and dorsal domain, respectively. Interestingly, *mirr* is ectopically expressed in the L3 vein in Mob^{1} wing disc. Therefore, Mob^{+} is required to repress *mirr* in the L3 vein precursor as well as in the ventral eye domain.

Mob might be a new gene nearby the *mirr* locus encoding a protein necessary for silencing *mirr* expression in the L3 vein and the ventral eye, or it may be a regulatory element within the *mirr* locus. We prefer the second possibility since *Mob* appears to be a *cis*-acting element. For instance, if *Mob* is a separate gene, Mob^{1} $mirr^{B1\text{-}12}/Mob^{+}$ $mirr^{B1\text{-}12}$ is expected to have the effect of ventral silencing because MOB protein will transact on $mirr^{B1\text{-}12}$, unless it is haploinsufficient. However, w^{+mC} was expressed in the ventral eye of these flies, indicating that the silencing effect occurs in *cis*. Furthermore, *Mob* fails to complement all *mirr* mutations. Therefore, *Mob* is likely to be an intragenic mutation in a regulatory region of the *mirr* locus. This is also consistent with the failure to find meiotic recombination between *Mob* and $mirr^{B1\text{-}12}$ despite our extensive effort (unpublished results). However, the possibility that *Mob* might be another gene nearby the *mirr* locus is not excluded.

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