u-shaped encodes a zinc finger protein that regulates the proneural genes achaete and scute during the formation of bristles in Drosophila

Yolande Cubadda,1 Pascal Heitzler,1 Robert P. Ray,2,3 Marc Bourouis,1 Philippe Ramain,1 William Gelbart,2 Pat Simpson,1 and Marc Haenlin1,4

1Institut de Génétique et de Biologie Moléculaire et Cellulaire (I.G.B.M.C.), Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale/Université Louis Pasteur (CNRS/INSERM/ULP), 67404 Illkirch Cedex, Communauté Urbaine de Strasbourg, France; 2Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138-2020 USA

The pattern of the large sensory bristles on the notum of Drosophila arises as a consequence of the expression of the achaete and scute genes. The gene u-shaped encodes a novel zinc finger that acts as a transregulator of achaete and scute in the dorsal region of the notum. Viable hypomorphic u-shaped mutants display additional dorsocentral and scutellar bristles that result from overexpression of achaete and scute. In contrast, overexpression of u-shaped causes a loss of achaete-scute expression and consequently a loss of dorsal bristles. The effects on the dorsocentral bristles appear to be mediated through the enhancer sequences that regulate achaete and scute at this site. The effects of u-shaped mutants are similar to those of a class of dominant alleles of the gene pannier with which they display allele-specific interactions, suggesting that the products of both genes cooperate in the regulation of achaete and scute.

A study of the sites at which the dorsocentral bristles arise in mosaic u-shaped nota, suggests that the levels of the u-shaped protein are crucial for the precise positioning of the precursors of these bristles.

[Key Words: Drosophila; u-shaped; zinc finger protein; bristle pattern; achaete; scute]

Received July 1, 1997; revised version accepted August 27, 1997.

The stereotyped positions of the large sensory bristles, or macrochaetae, of the Drosophila imago, provide a good model system for the study of pattern formation, and the analysis of genetic variants has allowed the identification of specific genes involved in the generation of this pattern (Lindsley and Zimm 1992). Each bristle organ is unique and develops within the imaginal disc from a single precursor cell, the sensory organ mother cell. Sensory mother cells (SMCs) arise as the result of a series of sequential steps. First, the competence to become a SMC is conferred to groups of cells at defined positions that prefigure the site of each future bristle. These cells are characterized by the localized expression of the proneural genes achaete (ac) and scute (sc), two members of the ac-sc complex (AS-C; Ghysen and Dambly-Chaudiere 1988; Campuzano and Modolell 1992). ac and sc encode transcriptional factors of the basic helix-loop-helix family first isolated in Drosophila whose activity confers neural potential to the cells (Villares and Cabrera 1987; Gonzalez et al. 1989). Loss of ac and sc causes a loss of all bristles on the thorax, whereas ectopic expression of these genes results in the formation of additional or ectopic bristles (Garcia-Bellido 1979; Balcells et al. 1988; Garcia-Alonso and Garcia-Bellido 1988). Neural potential is then progressively refined to a single cell, the future SMC within the proneural cluster by means of cell–cell interactions mediated by the neurogenic genes. This process is called lateral inhibition and involves an inhibitory signal from the nascent precursor that prevents the remaining cells of the cluster from becoming SMCs (for review, see Simpson 1990; Campos-Ortega and Jan 1991).

Proneural clusters arise in a precise spatial and temporal pattern that defines the positions at which each SMC and its corresponding macrochaete will arise (Romani et al. 1989; Cubas et al. 1991; Skeath and Carroll 1991). This dynamic expression pattern is controlled through the action of cis-regulatory sequences that modulate the expression of both ac and sc and that are scattered throughout the length of the AS-C (Ruiz-Gomez and Modolell 1987; Ruiz-Gomez and Ghysen...
1993; Gomez-Skarmeta et al. 1995). It is thought that in the imaginal epithelium, a prepatterning of asymmetically distributed factors regulate expression of ac and sc via these enhancer sequences.

Few factors regulating transcription of ac and sc have been identified, including the products of the genes hairy, extramacrochaetae, and those of the iroquois complex, araucan and caupolican. Hairy and Extramacrochaetae are negative regulators and encode products that belong to the family of proteins bearing helix-loop-helix motifs (Rushlow et al. 1989; Ellis et al. 1990; Garrel and M Odolell 1990). Hairy binds to ac upstream regulatory sequences and acts as a transcriptional repressor (Skeath and Carroll 1991; Ohsako et al. 1994; Van Doren et al. 1994), but Extramacrochaetae is thought to downregulate ac and sc function by protein association (Van Doren et al. 1991; Gomez-Skarmeta et al. 1995). The recently described araucan and caupolican proteins are transcriptional activators that belong to a novel family of homeoproteins and that regulate both sensory organ development and wing vein formation (Gomez-Skarmeta et al. 1996).

The gene panner (pnr) is also required for the spatial regulation of ac and sc in imaginal discs and encodes a protein with two functional domains—a zinc finger domain with homology to the vertebrate transcription factor GATA-1 and a domain comprising two putative amphipathic helices (Ramain et al. 1993). A number of alleles of pnr have been described causing either a loss of ac-sc expression at some sites and the corresponding loss of specific bristles, or ectopic expression of ac-sc and the formation of additional bristles (Heitzler et al. 1996b). A class of dominant alleles (collectively called pnrD), associated with lesions in the zinc finger domain, display an overexpression of ac and sc and the development of extra thoracic bristles. Here we describe mutants of the u-shaped (ush) gene, isolated during a screen for second-site modifiers of the pnrD phenotype (P. Heitzler, unpubl.). Alleles of ush causing a loss of function act as dominant enhancers of pnrD heterozygotes, resulting in an increase in the number of ectopic thoracic bristles. On the other hand, three copies of the wild-type ush gene suppress the pnrD/+ phenotype. This suggests that pnr and ush might act in the same developmental pathway to regulate the ac and sc genes.

A morphogenetic study of ush mutants and the molecular cloning of this gene is described. The bristle pattern of the viable recessive alleles of ush is similar to that of the pnrD alleles. We show that the additional bristles on the dorsal part of the thorax are associated with the overexpression of ac and sc in the corresponding proneural clusters. In contrast, ectopic expression of ush causes a loss of ac-sc expression that correlates with a loss of the corresponding dorsal bristles. ush encodes a novel zinc finger factor that has a role in the regulation of the spatial expression of ac and sc. The effects on the dorsal central bristles appear to be mediated through the enhancer sequences that regulate ac and sc expression at the presumptive dorsal central site in the thoracic imaginal disc.

Results

Allele-specific interactions between pnr and ush mutants

A number of mutant alleles at the pnr locus have been described including a class of dominant alleles (collectively called pnrD) associated with overexpression of ac-sc and additional dorsal central bristles (Ramain et al. 1993). This phenotype is similar to that of viable ush mutants and, furthermore, both ush hypomorphs and pnrD heterozygotes lack the postvertical bristle on the head (Heitzler et al. 1996b). The phenotype of flies heterozygous for pnrD mutants is strongly modified by the dosage of ush+ (Fig. 1). A single copy of ush+ causes a dramatic increase in the number of dorsal central bristles, whereas three copies suppress the pnrD phenotype entirely. This genetic interaction is restricted to the pnrD class of alleles, no other pnr mutants are sensitive to the amount of ush product (P. Heitzler, unpubl.).

ush is required for the pattern of bristles on the dorsal notum

Mutants in the gene ush and their embryonic recessive lethal phenotype were first described by Nüsslein-Volhard et al. (1984). In ush null embryos the germ band fails to retract and lateral fusion occurs between anterior and posterior ectoderm (R.P. Ray, unpubl.). A large number of mutant alleles of ush have been isolated and include hypomorphic mutants that affect the pattern of bristles on the head and thorax (P. Heitzler, unpubl.). Viable transallelic combinations of ush mutants display additional dorsal central and scutellar bristles on the notum and a loss of postvertical bristles on the head (Figs. 1 and 2B). They form an allelic series for the number of dorsal central (DC), scutellar (SC), and postvertical (PV) bristles (e.g., the number of bristles per hemithorax/head was found to be (DC) 4.59 ± 0.18, (SC) 2.44 ± 0.09, and (PV) 0.72 ± 0.08 for ushrev2/ush--; (DC) 5.44 ± 0.25, (SC) 2.59 ± 0.10, and (PV) 0.53 ± 0.09 for ushrev2/ush--; (DC) 6.27 ± 0.14, (SC) 2.67 ± 0.10, and (PV) 0.15 ± 0.06 for ushrev24/ush--; Wild-type flies invariably bear two DC, two SC, and one PV).

To look at the complete loss of function of ush on the notum, we produced clones of cells mutant for the null alleles ushVX22, ushTGR+1, and ushE6 (see Materials and Methods). All three resulted in similar phenotypes and affected the development of the dorsal half of the notum only, clones in the lateral part of the notum differentiated normally (Fig. 1). Clones extending into the scutellum fail to differentiate, generating large gaps in this region. Consequently, mutant scutellar bristles are never observed. Clones touching the dorsal midline are associated with a cleft in the thorax, whereas clones extending into the dorsal central area are associated with absence or abnormal positioning of the dorsal central bristles. From a study of 54 clones, the anterior dorsal central bristle was found to be missing in 22 cases (41%), the posterior dorsal central in 14 (26%; Fig. 2E). Nevertheless four dorsal central bristles were of the mutant genotype showing
that ush is not required for the construction of these bristles (Fig. 2F). In contrast, additional dorsocentral bristles were observed in only 17 cases (31%; 5 anterior and 12 posterior). Mutant bristles, when they do form, are never found at the correct locations characteristic of the wild-type flies, but are displaced to nearby positions.

Remarkably, in almost all cases of mosaicism in the dorsocentral area, the dorsocentral bristles formed by wild-type cells were also found to be displaced from their normal positions (Fig. 2D). Frequently, wild-type bristles are found on the mosaic border between mutant and wild-type cells. We counted 26 wild-type bristles in the dorsocentral region that were located precisely on the mosaic border. Furthermore, another 29 wild-type bristles, also displaced from their normal positions, were located a short distance away from the clone border outside the mutant territory. Therefore, there seems to be a nonautonomous effect of the mutant cells on the posi-

**Figure 1.** Schematic representation of the bristle patterns on the head and thorax of different mutant combinations of ush. The wild-type pattern is shown in a. In ush mutants, the dorsocentral bristles (DC) and the scutellar bristles (SC) of the thorax and the postvertical bristle (PV) on the head are the most frequently affected. The rest of the bristle pattern is normal. For each genotype, at least 30 hemithoraces were examined. Bristles present >50% of the time are represented as black dots (wild-type positions) and blue circles (ectopic bristles), those present 10%–50% of the time are represented as yellow circles, and those present <10% of the time as red dots. (a) ANP and PNP are anterior and posterior notopleural bristles, respectively. (b–d) Three viable combinations forming an allelic series for the number of DC and SC bristles of the thorax and the PV on the head (see text). ush<sup>rev18</sup> is a deficiency uncovering several genes, including ush, and will be called ush<sup>-</sup>. An allele specific interaction exists between ush and pnr<sup>d</sup> mutants (e–g). pnr<sup>d</sup>+/+ flies (f) display additional bristles (DC and SC) and loss of PV bristles (Ramain et al. 1993), a phenotype that is suppressed by three doses of ush<sup>+</sup> (e) or enhanced when only one copy of ush<sup>+</sup> (g) is present in these flies. The thoracic territories affected by the lack of ush function as revealed by clonal analysis (see text) are presented in h. The pink area denotes a territory where ush is required for cell viability and the green area denotes a territory where ush is required for the normal positioning of the DC bristles, part of this territory is also required for the fusion of the dorsal midline. When both ac and sc are nonfunctional, as in ac<sup>sc10-1</sup> flies, no bristles form whether or not ush is present (j and i).
tion at which wild-type bristles will form. Taken together, these results suggest that the position at which the dorsocentral bristles form may be dependent on the relative levels of ush product.

To investigate these observations further, animals mosaic for the viable hypomorphic allele ush<sup>rev24</sup> were generated. In these mosaics, wild-type ush<sup>+</sup> cells are juxtaposed to cells containing a reduced amount of Ush. In contrast, overexpression of ush leads to loss of the DC bristles (hs-ush is shown in C). Absence of ush function also affects the formation of DC bristles, as revealed in clones of cells mutant for null alleles (D–F). Clones of mutant cells were generated during the larval stages (see Materials and Methods for details and complete genotypes). The mosaic border of the clones is indicated by a black line (D–F). A clone mutant for ush<sup>TgR+1</sup> (D) shows the formation of wild-type DC bristles that are displaced from the normal site and have formed at the borderline separating wild-type and mutant cells. In large clones encompassing the entire dorso-central region, no DC bristles form (E). However, ush function is not required for the construction of bristles in this area because occasionally an additional mutant DC bristle develops (arrow in F). (G–I) Drawings of clones situated in the dorso-central area. (C) Bristles of the wild-type genotype that have formed at ectopic positions at the borders of the mutant clones; (●) the normal positions at which these bristles should have been situated.

ush is expressed in the dorsal part of the thoracic imaginal disc

To determine the location of ush transcripts in the thoracic epithelium, we performed in situ hybridization with a digoxigenin-labeled ush cDNA probe (see below) in late third-instar imaginal discs. Expression of ush appears to be restricted to specific domains (Fig. 3D). Staining is detected in territories corresponding to the future dorsal-most region of the thorax, as well as in part of the hinge region and the posterior region of the pleura. In the hinge region, ush expression is found in a domain comprising the sites of appearance of the anterior notal wing process and the proximal tegula, expanding up to the border where anterior and posterior notopleural bristles develop (ANP and PNP in Fig. 1). In the dorsal part of the notum, staining covers the site of appearance of the scutellar bristles and extends to the border of the site at which the dorsocentral bristles form. Therefore, in the notum, the area of ush expression corresponds well with the region where ush is required for normal development. In contrast, no apparent phenotype has been detected in the hinge and the pleura in ush mutants, suggesting that activity of the gene is not essential at these sites.

The additional bristles formed in ush hypomorphic mutants result from overexpression of ac and sc

Formation of the thoracic sensory bristles is known to result from expression of the ac and sc genes and so we investigated the relationship between ush and ac–sc. As the effects of ush are strongest on the two dorsocentral macrochaetes, we focused our analysis on these bristles.
Several lines of evidence suggest that the effects of ush are mediated by ac and sc. First, a synergism between ac–sc and ush is observed—animals heterozygous for both a deletion of ush and a deletion of the AS-C lack the posterior vertical bristles on the head (PV, see Fig. 1). Second, ac–sc mutants are epistatic over ush for the bristle phenotype—in the absence of ac–sc function, no sensory organs develop even in ush mutant flies (Fig. 1). This indicates that Ush functions before Ac and Sc. The additional macrochaetes seen in ush mutants may be therefore be attributable to overexpression of ac and sc.

To follow expression of ac and sc, we made use of the two transgenes ac–lacZ and scabrous (sca)–lacZ (Mlodzik et al. 1990; Martinez and Modolell 1991). The ac–lacZ transgene, which contains the 3.8-kb ac promoter region fused to the lacZ gene, expresses lacZ weakly in proneural clusters attributable to activation by the endogenous Ac and Sc proteins (Martinez and Modolell 1991; Gomez-Skarmeta et al. 1995; Fig. 3A). The sca–lacZ line contains a lacZ insert in the sca gene, which is known to be a direct target for Ac and Sc. It shows a pattern of expression similar to that of ac and sc in the thoracic discs and ectopic expression of either ac or sc has been shown to induce ectopic expression of sca (Mlodzik et al. 1990; Fig. 3E). For one allelic mutant combination, we also looked for an antibody against Ac and observed a similar expression pattern. We have also followed development of the SMCs using A101, a lacZ insert in the neuralized gene, which is expressed in all sensory organs precursors (Fig. 3I; Boulianne et al. 1991).

In hypomorphic ush mutants, the excess of dorsocentral bristles correlates with the segregation of an excess of SMCs (Fig. 3). Moreover, comparison between the dorsocentral region of wild-type and ush mutant discs, reveals a much larger territory of expression of ac–lacZ and sca–lacZ (Fig. 3B,F) in the mutants, which extends as far as the scutellum region. Altogether, these observations indicate that overexpression of ac and sc genes is responsible for the excess of bristles formed in the ush mutants.
Overexpression of ush leads to down-regulation of ac and sc and a loss of bristles.

The domain of expression of ush extends from the dorsal midline of the thorax to the border of the site of formation of the dorsocentral macrochaetes. Although the number and position of these bristles is affected strongly in ush mutants, ush expression is only detectable at a low level at the site of the dorsocentral precursors themselves, suggesting that the levels of ush product in this area may have a determining role. To test this, we have looked at the consequences on the development of these bristles of a higher level of ush expression.

To overexpress ush, we generated transgenic flies carrying the ush cDNA under the control of a heat shock promotor and expressed ush ubiquitously during the period of time when the bristle precursors are determined. Overexpression of ush in imaginal thoracic discs during the third-larval stage up to early pupariation (see Materials and Methods) leads to the loss of dorsocentral bristles (Fig. 2C). The precursors for these bristles could not be detected in corresponding discs stained for A101 (Fig. 3K) and levels of ac–sc activity were low as shown by the loss of LacZ expression of the ac–lacZ and sca–lacZ inserts (Fig. 3C,G). Using the GAL4–upstream activating sequence (UAS) system (Brand and Perrimon, 1993) we also expressed Ush in a large domain in the dorsal thorax corresponding to that of the pnr gene (Raimain et al., 1993). The GAL4 line pnr^MD237 (Calleja et al. 1996; Heitzler et al. 1996b) was used to drive UAS-ush expression in the dorsal half of the notum, and this resulted in a loss of sca–lacZ expression in the dorsocentral proneural cluster, a loss of the dorsocentral precursor cells and a subsequent loss of dorsocentral bristles (Fig. 3H,L). Therefore overexpression of ush in the dorsocentral territory of the notum prevents formation of the dorsocentral macrochaetes.

ush acts through the dorsocentral enhancer of ac and sc.

The results presented in the preceding section suggest that ush may regulate the expression of ac–sc at the dorsocentral site. The dorsocentral proneural cluster of ac–sc expression is known to depend on enhancer sequences (the DC enhancer) located 4.0–9.8 kb upstream of the ac transcription start (Gomez-Skarmeta et al., 1995). To test whether the effects of ush are dependent on these sequences, we have examined the consequences of both loss of function and overexpression of ush on lacZ expression driven by the DC enhancer, using the Dc–ac2 and DC–3.2 lines described by Gomez-Skarmeta et al. (1995). Expression of both of these transgenes is increased in discs mutant for hypomorphic ush alleles and extends over a wider area when compared with wild-type controls (Fig. 4B,E). In contrast, ectopic expression of ush (UAS-ush driven by pnr^MD237) resulted in a loss of the dorsocentral cluster of lacZ expressing cells in thoracic discs from the Dc–ac2 or DC–3.2 lines (Fig. 4C,F).
observations are consistent with a regulatory role for ush in the expression of ac and sc in the dorsocentral proneural cluster.

Molecular cloning of the ush locus

To isolate the ush gene, we made use of the enhancer trap line 1513 (L. Seugnet and M. Haenlin, unpubl.), which behaves as a weak ush allele (ush1513). DNA surrounding the ush1513 element was obtained by the plasmid rescue technique (see Materials and Methods) and the resulting fragments were used to probe cosmids and λ phage genomic libraries. The location of this DNA in the ush region (21C), was confirmed by hybridization to salivary gland polytene chromosomes (data not shown). Figure 5 shows a map of ~40 kb surrounding the ush1513 insertion (at position +0 kb on the map). Southern analysis revealed that two mutants generated by mobilization of the P[lacZ] insert, ushrev24 and ushrev18, were each associated with deletions starting from the P insertion point and expanding distally and proximally to the centromere, respectively. In ushrev24,
which is a viable hypomorphic allele displaying a phenotype of an excess of bristles, sequences from position +2 kb leftward on the map are deleted. The ush<sup>res24</sup> mutant is associated with a complete loss of function of ush and also other genes; it was found to be associated with a deficiency, sequences are deleted from position ~4 kb rightward on the map. Mapping of the inversion breakpoint of In(2)Tg<sup>+</sup><sup>+</sup>, another null allele of ush (see Materials and Methods) suggested that the ush transcription unit is situated at the position +26 kb (Fig. 5).

Furthermore, fragments extending from +16 kb to +35 kb were found to label the embryonic amnioserosa after in situ hybridization, which is known to be affected in ush mutants (P. Heitzler and R. Ray, unpubl.). These DNA sequences were used to probe a Northern blot from embryonic, larval, pupal, and adult stages. A poly(A)<sup>+</sup> transcript of ~4.7 kb was detected with peaks of expression during early embryonic stages, at 4–8 hr, and also in third-instar larvae, pupae, and weakly in adults (data not shown). These fragments were also used to probe an embryonic cDNA library prepared from 4- to 8-hr embryos (see Materials and Methods). From the four cDNAs isolated, we sequenced the longest one (pU4.3) together with the corresponding genomic regions. The deduced structure is shown in Figure 5. To confirm that the transcription unit that we identified corresponds to the ush gene, we performed germline transformation with a cosmid clone. The cosmid cos4 contains sequences extending from the breakpoint of ush<sup>res24</sup> (+2 kb) to the end of the transcription unit (+35 kb, see Fig. 5A) and therefore probably spans the entire transcription unit. No other transcription unit were detected. The transformed line obtained with this cosmid inserted on the second chromosome is able to rescue to viability the two lethal mutants ush<sup>res22</sup> and ush<sup>res1</sup>, demonstrating that cos4 contains the ush gene. However, the rescued flies show a bristle phenotype similar to the viable mutant ush<sup>res24</sup>, indicating that regulatory sequences located further upstream are required for complete wild-type function.

ush encodes a large putative zinc finger protein

The sequence of both genomic and cDNA pU4.3 clones revealed a single open reading frame (ORF) coding for 1191 amino acids with a relative molecular mass of 123 kD (Fig. 5B). The Ush ORF displays two types of repeated motifs, CCHH and CCHC, found in several transcription factors of the zinc finger family (Berg 1993). Apart from the typical arrangement of the two cysteines and histidines, no particular consensus is apparent for the CCHH motifs. Nevertheless, the first motif of this type (amino acids 281–301) shows a similarity to the second zinc finger motif of the ZFY transcription factor family (Fig. 6; Ashworth et al. 1989; Mardon et al. 1990; Palmer et al. 1990). The CCHC motifs have been described for several zinc finger proteins, but the role of this structure has not been elucidated. Finally, the Ush protein also contains an acidic domain in the amino-terminal part of the protein (amino acids 9–104), and several stretches of alanine residues (amino acids 421–431, 674–683, and 1107–1114). All of these characteristics suggest a role for Ush as a nuclear factor. Immunocytochemical
analysis of ush cDNA transfected Cos cells with an antibody against Ush revealed a nuclear localization (data not shown). During embryogenesis, Ush is also localized in the nuclei of the cells in which it is expressed (data not shown). Unfortunately, because of the poor sensitivity of our immunodetection technique, we were unable to confirm this nuclear localization in the wing disc.

Discussion

The ush gene is required during both embryonic and imaginal development of Drosophila, and encodes a large protein bearing structural zinc finger motifs and an acidic region similar to a number of known transcription factors. No significant homology to known molecules present in the databases was found, but some of the zinc finger motifs of Ush show homologies with several transcription factors. However, as these proteins contain many other zinc finger motifs with no homology to those of Ush, it is difficult to assign a specific function to these motifs. Recently, a protein has been isolated from the mouse that displays an overall structure very similar to Ush (Tsang et al. 1997). This protein, FOG, like Ush, bears an acidic domain located at the amino-terminal part of the protein and nine zinc fingers arranged in two clusters. Interestingly, the protein contains four CCHH and five CCHC motifs, like Ush. Furthermore, a strong homology is found between the CCHC motifs of FOG and Ush that display more than 50% identical amino acids depending on which zinc finger motif is compared (Tsang et al. 1997). Altogether, these data indicate that FOG and Ush, although not homologous, are sufficiently similar to suggest they comprise a new family of zinc finger proteins.

The zinc finger motifs suggest a putative DNA-binding function for the Ush protein. Using immunocytochemistry we were unable to establish the presence of Ush in the nuclei of thoracic disc cells, but a nuclear localization was observed in embryos. On the basis of genetic data ush regulates ac and sc expression via specific enhancer sequences, we have since investigated the possibility that Ush may bind these sequences directly. Gel shift assays with full-length as well as truncated Ush proteins purified from Escherichia coli or from SF9-infected cells with baculovirus did not reveal any binding specificity for the molecule to this putative target sequence. Therefore, Ush may function together with other intermediate factors in the regulation of this potential target sequence.

Our results suggest that ush has three roles in the thoracic imaginal disc, one for the viability of epithelial cells in a restricted area covering the scutellum, one for the dorsal midline closure, and the other for the development of dorsocentral and scutellar bristles in a larger territory, including both the scutellum and the dorsal half of the thorax. ush shows a restricted pattern of expression in the thoracic disc that correlates with those areas of the notum where its function is required. No variations in intensity of expression could be detected, however, that might account for a differential requirement in the scutellum for cell viability. Furthermore, two other areas of ush expression, in the hinge and pleural regions, do not correlate with any apparent visible phenotypes in clones of null alleles covering these territories. Different functions of ush in the different areas of the thorax may depend on other, differentially distributed factors.

In this study, we have concentrated on the function of ush in the development of the macrochaetes, in particular the dorsocentral bristles.

ush regulates ac and sc expression in the dorsocentral region of the notum

Viável hypomorphic loss-of-function alleles of ush display additional dorsocentral bristles. In contrast, overexpression of ush leads to a loss of these same bristles. Our results show that the effects of Ush are mediated through expression of the ac–sc genes. Analysis of double mutants showed that ac and sc act after ush during bristle development. Observations of hypomorphic alleles revealed that the formation of ectopic dorsocentral precursors is a consequence of overexpression of ac–sc in the dorsocentral region. The enhanced ac–sc activity is reflected by the increased expression of ac–lacZ and sca–lacZ. Moreover, this effect is mediated by the dorsocentral enhancer element described by Gomez-Skarmeta et al. (1995), as, lacZ expression driven by these sequences is stronger in mutant ush discs than in the wild type. Consistent with this, overexpression of Ush leads to the loss of the dorsocentral precursor cells and a concurrent loss of ac–sc expression, as well as a loss of the lacZ expression driven by the enhancer sequences. These results are in agreement with a negative role for ush in the regulation of the ac–sc genes at the dorsocentral site.

ush and pnr participate in the same regulatory process

The ush protein is unlikely to regulate the ac–sc genes directly and may function together with other factors. A good candidate to mediate ush function is the product of the gene pnr. A number of observations support this argument. First, a specific genetic interaction exists between a class of pnr mutants, pnrD, and ush mutants that act as dominant enhancers of pnrD. Second, the phenotype of hypomorphic ush mutants are remarkably similar to that of pnrD/+ flies, they display additional dorsocentral and scutellar bristles but a loss of postvertical bristles. Third, both Pnr and Ush regulate ac–sc expression in the dorsocentral proneural cluster and both appear to do so via the dorsocentral enhancer element (Gomez-Skarmeta et al. 1995; Haenlin et al., this issue). Taken together, these data suggest that Ush and Pnr function in the same developmental pathway to regulate ac–sc expression at the dorsocentral site. Recent work has revealed that Pnr is a transcriptional activator, that the Ush and Pnr proteins associate with one another, and that the activating function of Pnr is lost when it is associated with Ush (Haenlin et al., this issue). Further-
Cubadda et al.

more the pnrD proteins have a reduced ability to associate with Ush and almost completely resistant to the modulating effects of Ush (Haenlin et al., this issue).

A role for ush in the precise positioning of the dorsocentral bristles

Clones of cells mutant for null alleles in the dorsocentral region, have a mutant phenotype showing that the mutant cells act autonomously. Unexpectedly however, the mutant patches are frequently devoid of bristles. This is in contrast to the hypomorphic alleles, which result in the differentiation of additional bristles. These observations suggest that, although the amount of ush product is crucial for bristle development, bristles do not arise as a simple consequence of the presence or absence of the Ush protein. Rather, the levels of ush product may be involved in the precise positioning of the bristles. Two observations are relevant to this question. First, it is noteworthy, that in the wild-type disc the dorsocentral precursors arise at the very edge of the domain of detectable ush expression, at a site where expressing and non-expressing cells are likely to be adjacent (or where the levels of Ush may be reduced). Second, in mosaic thoraces where mutant and wild-type cells are juxtaposed, the bristles are never in the correct positions and the majority of them form precisely along the mosaic border. This observation holds whether the bristles are of the mutant or the wild-type genotype. These observations suggest strongly that bristles form at the edges of a boundary between high and low levels of Ush. We have also observed that under mild heat shock conditions, an increase of ush expression causes the development of additional dorsocentral bristles, at a position that would correspond to the border of the endogenous ush expression domain (data not shown).

There is an additional feature of the mosaics that is of importance. The position of the wild-type bristles is modified by the neighboring mutant cells, that is, the mutant cells have a nonautonomous effect on their wild-type neighbors. Therefore, in the case of the mosaics involving the null mutant clones, the majority of bristles that form are of the wild-type genotype and they are found in altered positions demonstrating that the wild-type cells have been induced to develop bristles at ectopic sites by the presence of neighboring mutant cells. Furthermore, not only do wild-type bristles form at the clone border, they are also displaced a short distance away from the border. The distance separating the marked mutant cells from the wild-type bristles is one, two, or occasionally three epidermal cells. It is of interest that similar observations have been made in mosaics of pnrD mutants (P. Heitzler and P. Simpson, unpubl.). This suggests that in the absence of Ush (or in the presence of the constitutively active PnrD), a diffusible factor may be generated. Therefore, at a border of ush− clones, such a hypothetical factor might diffuse into the neighboring ush+ cells. Interestingly, in mosaics involving hypomorphic alleles, bristles also form at the edge of the mutant clone borders, but in this case the bristles are mainly of the mutant genotype. Production of the postulated diffusible factor may well be stronger in the case of the null mutant clones than for the clones of the hypomorphic alleles. In this context it is of note that the gene wingless, which encodes a diffusible protein, is expressed in a stripe along the notum adjacent to that of ush, and that expression of wingless changes in both pnr and ush mutants (Calleja et al. 1996; Y. Cubadda et al., unpubl.). It has been shown that wingless expression in the notum is required for the normal development of the dorsocentral bristles (Phillips and Whittle 1993). Further studies are required to determine whether the postulated diffusible factor correspond to Wingless.

In conclusion, we suggest that Ush may function to modulate locally the levels of ac–sc to precisely define the position of the precursor. It is known that a group of about six cells in the proneural field express ac–sc to high levels and are competent to make the bristle precursor (Heitzler and Simpson 1991; Cubas and Modolell 1992). The choice of which cell will do so depends in part on lateral signaling mediated by the Notch signaling pathway (Heitzler and Simpson 1991). However, the position of the precursors from the dorsocentral proneural cluster appears to be invariant (Cubas and Modolell 1992), suggesting that specific cells are predetermined to become the SMcs. Local modulation of the Ac–Sc levels could provide a bias. A cell could gain an early advantage by expressing higher levels of ac–sc than its neighbors and therefore be more competitive for the neural fate (Heitzler and Simpson 1991; Heitzler et al. 1996a). Further studies are necessary to determine how Pnr and Ush cooperate in the regulation of ac and sc.

Materials and methods

Fly strains

The locus ush was first defined genetically by the two amorphic mutants ush1D209 and ush1A102 which are lethal at embryonic stages and affect the pattern of the larval cuticle (Nüsslein-Volhard et al. 1984). A large number of mutant alleles have since been isolated and a complete description of the genetics of these will be described elsewhere (P. Heitzler, R.P. Ray, Y. Cubadda, M. Haenlin, L. Seugnet, and P. Simpson, in prep.).

The transposon TE99(Z), located at 21C6 and carrying two adjacent (w+; rst−) units and an internal fold-back sequence (Ising and Block 1981, 1984) was found to be the direct cause of a viable leaky ush mutant phenotype (ushTE99Z). The ushW42 mutant is a spontaneous white (SW) derivative of ushTE99Z that exhibits a normal cytology at 21C6, but displays a strong repressive viable adult phenotype. The transposon TE93(R) (Ising and Block 1981, 1984) was used as an ush+ duplication (P. Heitzler, unpubl.).

ush1511 behaves as a hypomorphic allele of ush and carries a P[Ac2, w+] transposon inserted next to the ush gene. Excision of the P[Ac2, w+] resulted in either wild-type ush+ flies, or in a series of ush recessive loss-of-function mutants such as ush1w18, ush2w16, and ushVw04.

ushTgR+1, ushVX22, and ushF6 correspond to amorphic mutants based on their genetic behavior. They both display the same embryonic phenotype as deletions when homozygous (P. Heitzler, unpubl.). Furthermore, embryos homozygous for ushTgR+1, ushVX22, or ushF6 show no detectable Ush protein.
expression using a monoclonal antibody (2us1D5) directed against the ush product. The pnrΔD1 mutant is described in Ramain et al. (1993).

The transgenic line B17 (ac-lacZ) harbors 3.8 kb of the ac promoter region fused to the lacZ gene (for details, see Martinez and Modolell 1991).

The transgenic lines DC-3.2 and DC-ac2 harbor the 5.7 kb EcoRI fragment that contains the dorsoventral enhancer fragment fused to 3.7Sc-lacZ (3.7 kb of the Sc promoter region fused to the lacZ gene) or to 0.8ac-lacZ (0.8 kb of the ac promoter region fused to the lacZ gene) (for details, see Gomez-Skarmeta et al. 1995).

Flies were raised on standard Drosophila medium at 25°C. More than 30 hemithoraces were examined for each of the genetic combinations presented in Figure 1.

Clonal analysis

Mutant clones were produced by hs-FLP/FRT-induced mitotic recombination (Golic and Lindquist 1989; Golic 1991) following procedures described in Heitzler et al. (1996a). Clones were induced in flies of the following genotypes. (1) y FLP1/y; ushV222 P(+)y;25F ckCH52 FRT40A/y; y FLP1/y; TdG+1 P(+;ry+)25F ckCH52 FRT40A/ P(m,w+)+21D P(m,w+)+36F; (2) y FLP1/y; TdG+1 P(+;ry+)25F ckCH52 FRT40A/ P(m,w+)+21C P(m,w+)+36F FRT40A; (3) y FLP1/y; Df(2L)ushw+ P(+)y;25F ckCH52 FRT40A/ P(m,w+)+21C P(m,w+)+36F FRT40A; and (4) y FLP1/y; ushV222 P(+)y;25F ckCH52 FRT40A/ P(m,w+)+21C P(m,w+)+36F FRT40A.

DNA sequencing

Sequences were obtained using the dideoxy chain termination method as described in Ramain et al. (1993). A cDNA insert of 4.7 kb (KpnI–HindIII fragment) was cloned in pBluescript II SK+ (Stratagene) and directional deletions were generated using the exonuclease III (Stratagene kit). Sequence data were collected for both strands using the M13 universal primer as well as internal synthetic oligonucleotides. The intron/exon structure, as well as the genomic organization of the ush locus, were determined first by hybridization of the cDNA on genomic sequences to localize the exons, and then by sequencing the corresponding genomic fragments using internal synthetic oligonucleotides. A search for homology was performed using the University of Wisconsin GCG software packages (Devereux et al. 1984). In vitro transcription and translation of the cDNA in rabbit reticulocyte lysates and SDS-PAGE analysis revealed a product (Ush) migrating at 200 kD, which confirmed the predicted ORF. Compared with the predicted 123 kD, the difference observed might be attributable to the presence of putative sites for glycosylation in the Ush protein sequence (Hirschberg and Snider 1987).

Expression of ush transcripts in situ

Digoxigenin-labeled DNA was synthesized according to the Boehringer Mannheim protocol and hybridized to imaginal discs using the method of Cubas et al. (1991), modified from Tautz and Pfeifer (1989).

Overexpression of Ush

The cDNA containing the entire ush ORF was subcloned in the plasmid pCAsper-hs (a gift from C.S. Thummel, University of Utah, Salt Lake City) and in pUAST (Brand and Perrimon 1993) using appropriate restriction sites. These plasmids were used to transform embryos of a w1118 stock and homozygous lines were established for the second and third chromosomes. UAS-ush lines were crossed to several lines expressing GAL4, but only crosses with the pnrMD237 Gal4-expressing line (Calleja et al. 1996; Heitzler et al. 1996b) gave viable adult progeny. Early third-instar larvae were subjected throughout two days to a heat treatment regime of 30 min at 37°C followed by 2 hr at 25°C.

Acknowledgments

We thank Cathie Carteret, Serge Vicaire, and Claudine Ackerman for excellent technical assistance; Adrien Staub and Frank Ruffenach for oligonucleotide synthesis; Yves Lutz for making the Ush antibody; Juan Modolell, Marek Modzik, Ginés Morata, and the Drosophila stock centres at Bowling Green and Umea for mutant strains; and our colleagues at the I.G.B.M.C. for thoughtful discussions throughout the course of this work. We are grateful to Stuart Orkin for sharing unpublished information with us and Juan Modolell and especially Pilar Cubas for their help with the technique of in situ hybridization in imaginal discs. M.H. visited the laboratory of Juan Modolell as a recipient of a short-term European Molecular Biology Organi-

GENES & DEVELOPMENT 3093

u-shaped, a zinc finger protein
References


Devereaux, J., P. Haeberli, and O. Smithies. 1984. A comprehen-


u-shaped encodes a zinc finger protein that regulates the proneural genes achaete and scute during the formation of bristles in Drosophila


Genes Dev. 1997. 11:
Access the most recent version at doi:10.1101/gad.11.22.3083

This article cites 53 articles, 25 of which can be accessed free at:
http://genesdev.cshlp.org/content/11/22/3083.full.html#ref-list-1

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.