our models; Arg^{16} is conserved betwen HNP-3 and NP-3b, and in the HNP-3 crystal structure this Gu forms a hydrogen bond with Thr^{18} (equivalent to Asp^{18} of NP-3b). In each of our three hypothetical membrane-bound models the Arg^{16} side chain can be repositioned so that its methylene groups contact lipid tails while its Gu group binds lipid head groups. Perhaps in NP-3b the Arg^{16} Gu maintains a salt bridge with Asp^{18} within the hydrophobic lipid environment. Such intramembrane salt bridges have precedence (*32*). Despite the high degree of defensin sequence variation, the flexibility of Arg side chains and plasticity of the membrane suggests that the different defensins could all interact with membranes in an identical manner.

All three of the hypotheses are consistent with the observation that a membrane potential is required for defensin activity (*8, 9*). In the wedge model the net negative charge on the inside of the cell drives the cationic wedge into the bilayer. In the pore models the potential is required to pull some of the Arg side chains completely across the membrane. All three models also rationalize the observed biphasic binding kinetics (*5*), in which the first step is predominantly electrostatic (Arg side chains with head groups) and the second of a more hydrophobic nature with lipid functions that are initially cryptic (hydrophobic dimer surface with lipid tails).

Defensin shares more in structural characteristics with small toxins that act by binding to specific receptor proteins than with other lytic peptides. Defensin's overall dimensions, positive charge, β sheet, and disulfide bonds are reminiscent of various snake, scorpion, and spider toxins (*33*) that function not by permeabilizing the membrane, but by binding molecules such as the acetylcholine receptor. Although similar to these, the defensin structure is quite different from other membrane-permabilizing peptides. The constrained, disulfide cross-linked structure, common to defensins and the small toxins, may reflect a requirement to maintain a stable and compact conformation to avoid digestion by proteases.

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20. Secondary structure was defined with the program DSSP (*21*). The first β strand, residues 4 to 6, is followed by a type VI turn (*22*) formed by residues 6 to 9, the third residue of which, Pro^8, is preceded by a cis peptide bond. Residues 9 to 11 are extended but without backbone hydrogen bonds. Then residues 11 to 14 form a type II turn with a hydrogen bond between 11 O and 14 N. At residue 15, the chain continues with a long β strand and then forms a type I′ hairpin at residues 22 to 25. The final β strand consists of residues from Gly^{24} through the carboxyl terminus. The β sheet is twisted in the usual sense (*23*), and this twist is exaggerated by a β bulge (*24*) formed by residues 17, 18, and 29.
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26. The GPNP sequence does not seem to be an exception: the Thr and Tyr that replace Arg^6 and Glu^{14} in GPNP do not seem to disrupt the structure, but rather fill the same volume as the Arg and Glu side chains in HNP-3; also a hydrogen bond can probably be formed between GPNP Thr^6 $O\gamma^1$ and Tyr^{14} $O\eta$.
27. NMR studies on NP-5 (*28, 29*), which show a β hairpin formed by residues 19 to 28 and a type I′ turn formed by residues 22 to 25, in general agreement with the HNP-3 crystal structure. The crystal structure of HNP-3 differs, however, from the NMR structure in details. For example, the crystal structure includes a third β strand formed by residues 4 to 6. This strand has not been described for NP-5, although inspection of stereo figures in (*29*) indicates that the NMR conformation in this region is similar to that in HNP-3 crystals.
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Cross-Regulatory Interactions Between the Proneural *achaete* and *scute* Genes of *Drosophila*

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The *achaete* (*ac*) and *scute* (*sc*) genes of *Drosophila* allow cells to become sensory organ mother cells. Although *ac* and *sc* have similar patterns of expression, deletion of either gene removes specific subsets of sensory organs. This specificity was shown to reside in the peculiar regulation of *ac* and *sc* expression. These genes are first activated in complementary spatial domains in response to different cis-regulatory sequences. Each gene product then stimulates expression of the other gene, thus generating similar patterns of expression. Therefore, removal of one gene leads to the absence of both proneural gene products and sensory organs in the sites specified by its cis-regulatory sequences.

THE CUTICLE OF *DROSOPHILA* CARries many sensory organs (SOs). The *achaete* (*ac*) and *scute* (*sc*) genes are necessary for cells to become sensory organ mother cells (SMCs) (*1*). In the imaginal discs that give rise to the adult epidermis, *ac* and *sc* are expressed in groups of cells called the proneural clusters, which delimit the sites where SMCs will develop (*2*). Although these genes are expressed in similar

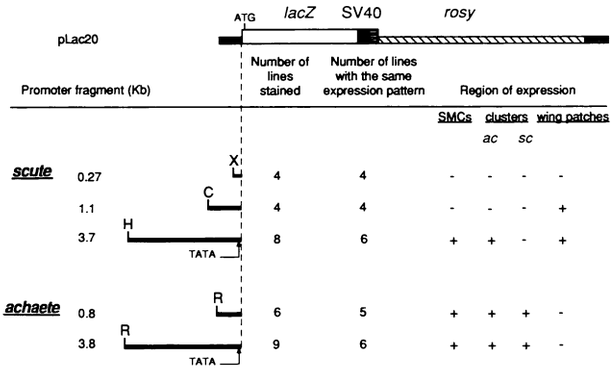

Fig. 1. Constructs with different lengths of the *ac* or *sc* promoter regions fused to *lacZ* from *Escherichia coli* used to transform *Drosophila*. Top, structure of pLac20 vector, which contains a Not I cloning site upstream from *lacZ* and the SV40 polyadenylation signals (*20*). C, Cla I; H, Hind III; R, Eco RI; and X, Xho I. Assayed lines resulted from independent integration events. Sites of *lacZ* expression are those of the consensus pattern for each type of construct (*21*). Clusters correspond to SOs that are thought to depend on *ac* or *sc* for development.

patterns, they have very different cis-regulatory regions. The cis-regulatory region of *sc* is complex and extends for over 50 kb (*3–5*), while that of *ac* is simpler and occurs within 0.8 kb upstream of the structural gene (*3*). To analyze how *ac-sc* expression is regulated, we have examined in wing imaginal discs of transformed flies the expression of a *lacZ* gene driven by different *ac* or *sc* promoter fragments (Fig. 1).

A 3.7-kb *sc* promoter fragment drove *lacZ* expression in SMCs of third instar wing discs (*6*) (Fig. 2). However, it did not induce expression in neighboring cells that constitute the proneural clusters (*2*). Exceptions to this are the clusters associated with the dorsocentral (DC) and posterior supraalar (PSA) macrochaetae precursors (Figs. 2 and 3A). Hence, this promoter fragment has sequences that allow specific *sc* activation in SMCs.

The 1.1- and 3.7-kb *sc* promoters fragments, but not that of 0.27 kb (Fig. 1), induced strong *lacZ* expression in five patches on the wing primordium (Fig. 2, top row). Although some patches encompassed areas where wild-type *sc* is expressed [for example, the presumptive third vein (patches 1 and 3) and the base of the anterior wing margin (*2*) (patches 4 and 5)], these patches are much larger than the areas of wild-type *sc* expression. This indicates that the promoter fragments contain sequences for specific *sc* activation in these regions of the wing disc, although they lack other sequences that restrict it to the wild-type areas. Genetic data have suggested the presence of control elements for wing sensilla in the *sc* promoter (*4*).

Expression mediated by the 3.7-kb *sc* promoter fragment depended on endogenous *ac* and *sc*. Removal of *sc* eliminated expression in SMCs that give rise to *sc*-dependent SOs (Fig. 2, sc^-), probably because these cells do not appear under these conditions. The SMCs that remained corresponded to the DC and PSA macrochaetae and the dorsal and ventral HCV sensilla campaniformia, all of which require *ac* for development (*4, 7*). Removal of *ac* eliminated *lacZ* expression in these cells (Fig. 2; ac^-, positions marked by arrowheads). Most importantly, the absence of *ac* strongly decreased expression in the DC-PSA clusters (Fig. 3A), which suggests that *ac* promotes wild-type *sc* expression in this region. In the absence of both *ac* and *sc*, *lacZ* expression remained only in the wing patches and weakly in the DC-PSA region (Fig. 2).

The 0.8- and 3.8-kb *ac* promoter fragments induced similar patterns of *lacZ* expression in SMCs and their associated clusters (Figs. 2 and 3A; SMCs are recognizable because of their stronger staining). The patterns of expression (Fig. 2) were similar to those of the endogenous *ac* gene (*2*). Activation of the *ac* promoter was also largely dependent on the endogenous *ac* and *sc* genes. Thus, removal of *ac* eliminated expression in SMCs that corresponded to the *ac*-dependent SOs and decreased expression in the DC and PSA clusters (Figs. 2 and 3A; ac^-, bottom row). The absence of *sc* eliminated expression of *lacZ* in the complementary set of SMCs and associated clusters (Fig. 2, sc^-, bottom row). When both *ac* and *sc* were eliminated *lacZ* expression was still observed in the wing pouch, in an area that covered the DC-PSA region, and in the anterior part of the presumptive notum (Fig. 2). These results suggest that many of the sequences required for wild-type *ac* expression in the wing disc are present in the 0.8-kb fragment.

Our findings indicate that *ac* stimulates expression of the endogenous *sc* gene at a few sites, while the reciprocal stimulation occurs at many more sites. To confirm these interactions, endogenous *ac* and *sc* mRNA distributions were examined in sc^- and ac^- backgrounds, respectively. Each mRNA was only present in those sites where *lacZ* expression appeared in the same genetic backgrounds (Fig. 3B) (*8*). In an earlier study, the difficulties associated with the interpretation of patterns from disc sections prevented the detection of these modifications (*2*).

The above results suggest that the patterns of expression of *ac* and *sc* are controlled by the cis-regulatory sequences of both genes. Indeed, these sequences direct transcription of the respective gene in distinct sites. Once activated, each gene stimulates expression of the other, yielding overlapping patterns of expression (displayed graphically in Fig. 4). *sc* has a more complex cis-regulatory region than *ac*. This difference in complexity is consistent with *sc* initially

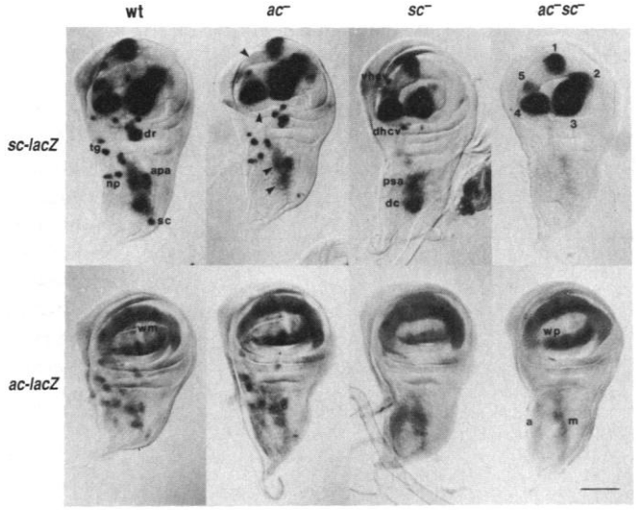

Fig. 2. *lacZ* expression driven by either the 3.7-kb *sc* (*sc-lacZ*, top row) or the 3.8-kb *ac* promoters (*ac-lacZ*, bottom row) in wild-type (wt), ac^- ($Df(1)y^{3PL}sc^{8R}$), sc^- ($Df(1)sc^{8L}sc^{4R}$), and ac^-sc^- ($In(1)sc^{10.1}$) wing discs; sc, dhcv, vhcv, np, and apa correspond to isolated SMCs, while dr corresponds to a group of SMCs [for nomenclature see (*22*)]. The X-gal stained cells appear artifactually enlarged. Anterior wing margin (wm) SMCs barely expressed *lacZ*. Expression of the *ac* promoter in the wing pouch (wp), seeming to reveal the presumptive wing veins, was not observed with the *ac* mRNA; a and m show anterior and medial regions of the presumptive notum. Bar, 100 μm (*23*).

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being expressed in more sites than *ac*. Because the Ac and Sc proteins are members of the helix-loop-helix (HLH) family of transcriptional regulators (*9*), the cross-stimulation between *ac* and *sc* may be directly mediated by their products, which may bind to the reciprocal regulatory regions. In fact, DNA binding assays in vitro revealed the presence of several binding sites for *sc* and other HLH proteins in the 0.8-kb region of the *ac* promoter (*10*).

Expression of *ac* and *sc* is regulated at several levels. First, each gene is activated in distinct sites by as yet uncharacterized factors. Second, each gene regulates expression of the reciprocal gene. Third, each gene probably stimulates its own expression. In the case of *ac*, the self-stimulation occurs in the DC-PSA area. Moreover, generalized *ac* expression in the *Hairy-wing*[1] mutant promotes ubiquitous expression of the 0.8-kb *ac* promoter (*11*). Self- and cross-stimulation of *ac* and *sc* may be interferred with by the HLH proteins encoded by the *hairy* and *extramacrochaetae* genes (*12, 13*), negative regulators of the *ac-sc* functions (*14, 15*). These proteins may complex with the Ac and Sc proteins and alter their putative DNA binding activities. In addition, *sc* and, possibly, *ac* are specifically activated in SMCs. In fact, SMCs have higher amounts of *ac-sc* mRNA and protein than all the remaining cells of the proneural cluster (*8*). This regulation may be used to maintain SMC commitment or to activate the developmental program for SO differentiation (*2*). A similar function has been proposed for MyoD in myoblast differentiation (*16*).

The phenotypes of *ac* and *sc* mutations suggest that most SOs require either *ac* or *sc* for development (7), while other data indicate that the same SOs are not specific for either gene product (*3, 17–19*). Our results propose an explanation for this paradox. That is, *ac* and *sc* mutations primarily interfere with the expression of the respective gene, but due to their cross-stimulation the expression of both genes is affected. Therefore, the absence of an SO that appears to depend on one of these genes can be due to the loss of either Ac, Sc, or both gene products. This agrees with the observation that *sc* can promote development of SO previously thought to be *ac*-dependent (*3, 17–19*).

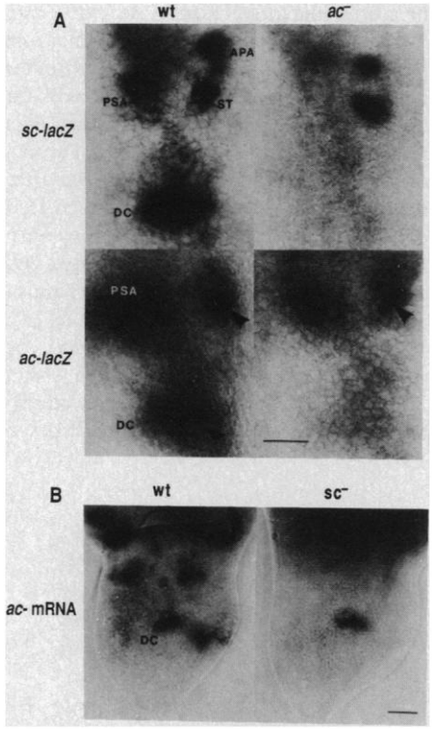

Fig. 3. Presumptive notum region of wild-type and different mutant discs that show *ac-sc* cross-regulation and *ac* self-stimulation. (**A**) *lacZ* expression driven by the 3.7-kb *sc* (*sc-lacZ*, top row) or the 3.8-kb *ac* (*ac-lacZ*, bottom row) promoters in the central area of the presumptive notum of wild-type (wt) and *ac*⁻ (*Df(1)y*^{3PL}*sc*^{8R}) wing discs. The area shown corresponds to the sites where the DC, PSA, APA macrochaetae, and sensilla trichoidea (ST) precursors arise. In the bottom row, arrowheads indicate SMCs. Bar, 20 μm. (**B**) Distribution of *ac* mRNA in the presumptive notum region of a wild-type and *sc*⁻ (*Df(1)sc*^{8L}*sc*^{4R}) wing disc. The absence of *sc* abolished *ac* expression, except in the DC cluster. There was no expression in the PSA area; this occurs in younger discs (*8, 11*), but the stability of β-gal allows its detection in mature discs (Figs. 2 and 3A). Apparent general labeling in upper part of pictures corresponds to nonspecific staining of the peripodial membrane. Bar, 20 μm (*24*).

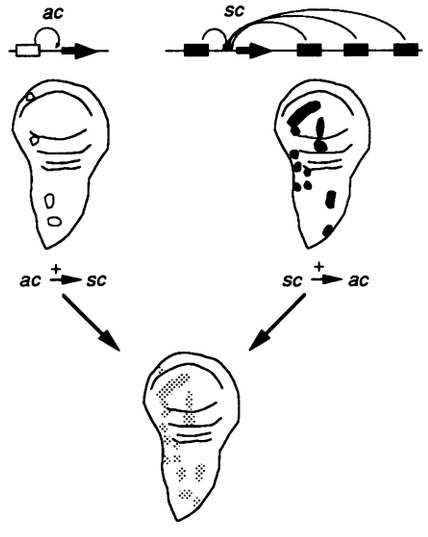

Fig. 4. Model for generating similar patterns of *ac* and *sc* expression. Top, *ac* in response to its cis-regulatory sequences (open rectangle) is initially expressed in a small number of sites (open patches on left wing disc). *sc*, driven by more complex regulatory sequences (filled rectangles) is expressed in a larger number of sites (right disc). *ac* and *sc* are expressed in different sites, which suggests they have different regulatory sequences. Bottom, cross-activation between *ac* and *sc* allows expression of both genes in all sites (hatched patches in bottom disc).

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6. These cells are SMCs or their descendants, because they are the same cells that express *lacZ* in the A37 and A101.1F3 transformants [A. Ghysen and C. O'Kane, *Development* **105**, 35 (1989) and (*11*)] and later become morphologically recognizable SOs (F. Huang, C. Dambly-Chaudière, A. Ghysen, *Development*, in press). SMCs are assigned to specific SOs according to the wing disc fate map [P. J. Bryant, *J. Exp. Zool.* **193**, 49 (1975)] and positions in the everting wing [M. A. Murray, M. Schubiger, J. Palka, *Development* **104**, 259 (1984)].
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23. Discs were either fixed with glutaraldehyde (0.5%) and stained with X-gal (0.2%) for 12 to 14 hours at 37°C (*2*) (top row) or fixed with methanol, reacted with antibody to β-galactosidase, and stained with secondary antibody coupled to horseradish peroxidase (bottom row).
24. Hybridization in situ was performed with a digoxygenin-labeled *ac* probe, as described [D. Tautz and C. Pfeifle, *Chromosoma* **98**, 81 (1989)]. Images were obtained with a Zeiss laser-scanning microscope.

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