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Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

The Complex Tale of the *achaete–scute* Complex: A Paradigmatic Case in the Analysis of Gene Organization and Function During Development

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ABSTRACT

The *achaete–scute* gene complex (*AS-C*) contains four genes encoding transcription factors of the bHLH family, *achaete*, *scute*, *lethal of scute*, and *asense* located in 40 kb of DNA containing multiple *cis*-regulatory position-specific enhancers. These genes play a key role in the commitment of epidermal cells toward a neural fate, promoting the formation of both sensory organs in the peripheral nervous system (bristles) of the adult and of neuroblasts in the central nervous system of the embryo. The analysis of the *AS-C* initially focused on the variations in positional specificity of effects of *achaete* (*ac*) and *scute* (*sc*) alleles on macrochaete bristle pattern in the Drosophila adult epidermis, and from there it evolved as a key entry point into understanding the molecular bases of pattern formation and cell commitment. In this perspective, we describe how the study of the *AS-C* has contributed to the understanding of eukaryotic gene organization and the dissection of the developmental mechanisms underlying pattern formation.

PATTERN formation consists of the generation of constant distributions of cell types in a developing tissue or organism. The analysis of the causal mechanisms underlying pattern formation has had a major impact in developmental genetics, due in part to the identification of genetic variants affecting the formation of sensory organs at specific spatial positions in the thorax and head of the fruit fly. In particular, the study of the *achaete–scute* gene complex has provided the bulk of information and concepts about gene organization, the spatial regulation of gene expression, the genetic and cellular mechanisms of cell commitment, and, more recently, the developmental bases of the evolution of both the genes and the patterns they determine. In this *Perspectives* we summarize some of the key aspects of the *achaete–scute* complex that have made a significant contribution to the understanding of the developmental mechanisms regulating pattern formation. We summarize the particular characteristics of *achaete* and *scute* alleles that made them attractive from the genetic point of view, the information gained by the molecular analysis of the genes, and the different aspects of bristle pattern formation that made the study of the *achaete–scute* complex a paradigmatic case of the analysis of developmental genes and the process they regulate.

GENETIC COMPLEXITY OF *scute* AND *achaete* MUTATIONS

The story began with the variations in positional specificity of *achaete* (*ac*) and *scute* (*sc*) mutations in the Drosophila adult epidermis, and, as we shall see, it progressed to identify crucial roles for the wild-type genes in neural development. At the time of their discovery, genes were just hereditary factors whose allelic variants allowed their mapping to chromosomes. The functional nature of these genes could be inferred only from the phenotype of their mutant alleles. For William Bateson, at the beginning of the 20th century, mutant alleles corresponded to the loss of function, but this idea started to be reconsidered when noncomplementing multiple alleles in the same gene appeared. For enzyme coding genes, this notion was understood as partial failures of a basic enzymatic function, *e.g.*, in eye pigment formation. Multiple alleles in the *white* gene, leading to varied tones of red, were more difficult to explain. It was found later that they were related to mutations in functional domains of a carrier protein

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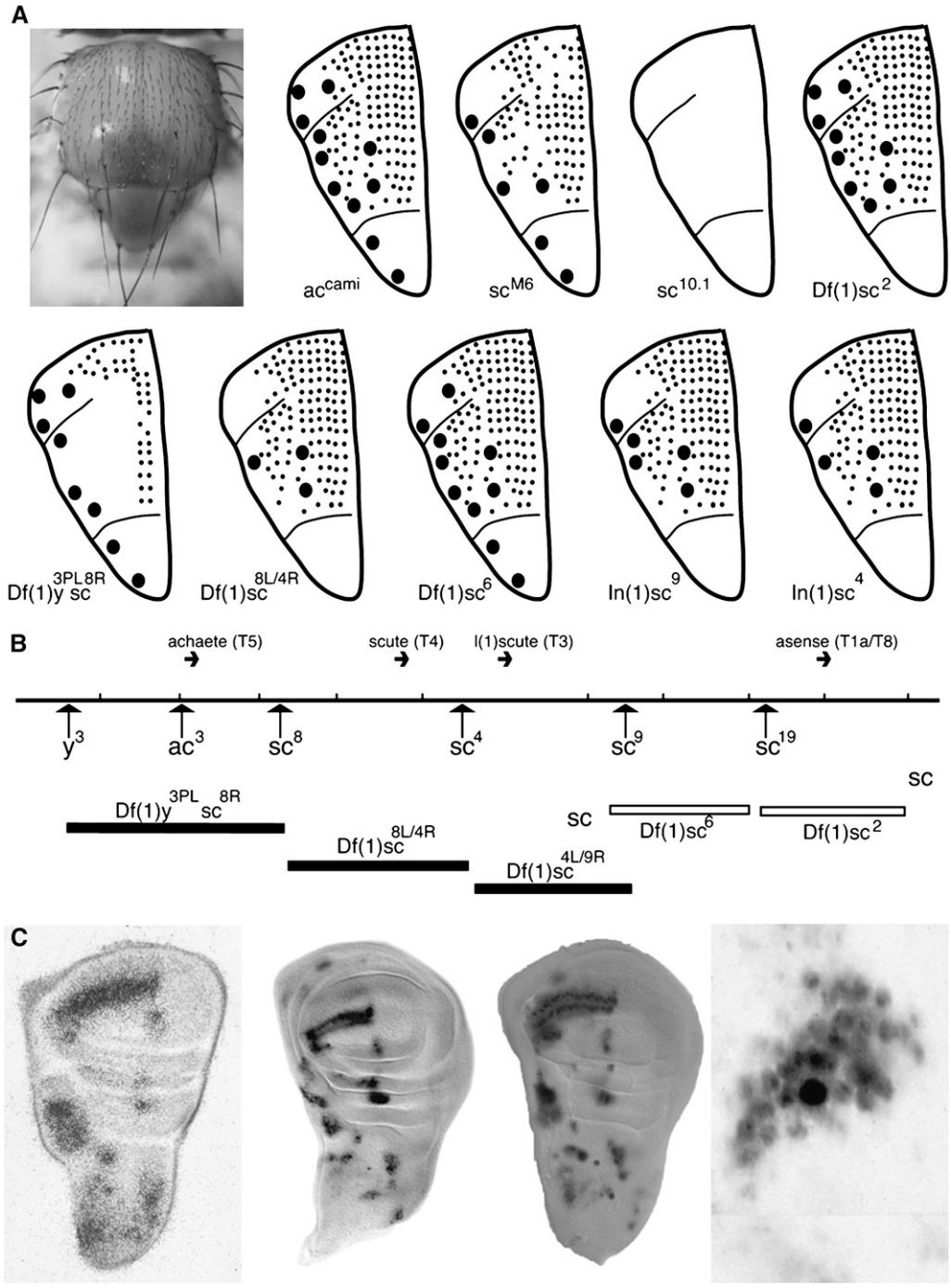

FIGURE 1.—(A) Top row from left to right: photograph of the fly thorax (courtesy of J. Modolell) and representations of the bristle phenotype in the left hemithorax of the point null alleles (ac^{cami} and sc^{M5}), the $sc^{10.1}$ double mutant, and the *asense* deficiency [*Df(1)sc2*]. Bottom row: representation of the bristle phenotype in the left hemithorax of the synthetic deletions for *achaete* [$Df(1)y^{3PL}sc^{8R}$] and *scute* [$Df(1)sc^{8L9R}$] and the *scute* alleles sc^6, sc^9, and sc^4. Note that the deficiency sc^6 has a much weaker phenotype than $In(1)sc^9$. (B) Representation of the achaete–scute complex, indicating the coding regions T5 (*achaete*), T4 (*scute*), T3 (*lethal of scute*), and T1a/T8 (*asense*) (horizontal arrows); the position of representative breakpoints (y^3, sc^8, sc^4, sc^9, and sc^{19}; vertical arrows); the deletions constructed from these breakpoints ($y^{3PL}sc^{8R}$, sc^{8L4R}, sc^{4L9R}; solid bars); and two proximal deficiencies, sc^6 and sc^2 (open bars). (C) From left to right, original photographs (courtesy of J. Modolell and S. Campuzano) of a wing disc section hybridized with a T4 radioactive probe, whole-mount wing discs stained with anti-Ac antibody or hybridized with a T5 probe labeled with digoxigenin, and high magnification of the dorsocentral proneural cluster stained with anti-Ac antibody.

displaying distinct affinities for different eye pigments. The allelic series of *achaete–scute* mutants defied a quantitative, lineal interpretation of the function of the genes in the terms suggested by H. J. Muller (amorphs, hypomorphs, and hypermorphs) to classify mutations on the basis of the results of genetic tests (MULLER 1932). Thus *ac* alleles showed specificity for the removal of microchaetae ("hairs" at the time) and some macrochaetae ("bristles") of the notum. The *sc* alleles eliminated only a subset of macrochaetae, those *not* affected by *ac* mutations (see Figure 1A). Some *sc* alleles behaved as noncomplementing in certain macrochaetae positions, but other *sc* alleles with different pattern specificities would complement for the positions not affected by these individual alleles. The positions of affected macrochaetae in individual alleles and allelic combinations followed a topological order ("seriation") that was clearly nonlinear in the thorax, but discontinuous. The colleagues of Muller in Moscow (A. S. Serebrovsky, N. P. Dubinin, and A. A. Prokofieva, *et al.*) designated these *sc*

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alleles step-alleles (Treppen allelomorphism in the original) (Agol 1931; Sturtevant and Schultz 1931; Dubinin 1932; Muller and Prokofyeva 1935).

The key point was that different alleles showed specificity in the positions of chaetae they affected. *ac–sc* alleles are readily inducible by x-ray irradiation and easily detected by changes in the otherwise constant pattern of thoracic chaetae. These alleles could not be mapped meiotically, because meiotic recombination does not occur in the tip of the X chromosome. This prevented the study of possible *cis*-effects among different alleles, unless they were induced in mutant chromosomes, as, for example, the $sc^{10.1}$ allele (see Figure 1A and below). With the increase in the number of alleles of *sc*, in particular, new combinations of affected positions continued to appear. This situation was a genetic challenge for many exceptional geneticists (*e.g.*, Alfred Sturtevant, Curt Stern, and Hermann Müller). It was also a challenge for developmental geneticists: it presented an opportunity to confront the fundamental problem of how "position" is encoded in the genome.

Many of the induced *sc* mutations were associated with chromosome breakpoints with one break in the "*ac–sc*" region (distal tip of the X chromosome) and another in the centromeric heterochromatin or in the euchromatin of the X chromosome (chromosomal inversions), or in any other autosomic arm (chromosomal translocations). Raffel and Müller (1940) used X chromosome inversions to generate deficiencies (loss of a chromosomal segment) and duplications of chromosomal segments in the *as–sc* region (the so called left–right test). This test revealed the existence of the "*ac*" region, the "*sc*" region, and a "*lethal of scute*" (*l'sc*) region located between *sc* breakpoints whose deletion was lethal (Figure 1, A and B). The results of a similar approach extending this test to more breakpoints, as well as to internal and terminal deletions and duplications constructed using autosomal translocations, confirmed the existence of the *l'sc* function and uncovered certain symmetries in the phenotypes of deletions at both sides of *l'sc* (named *sc*-α and *sc*-β) (Garcia-Bellido 1979). Interestingly, the phenotype of the breakpoints was more extreme the closer they mapped genetically to *l'sc*. In addition, certain duplications showed a phenotype in which extra chaetae differentiated in novel positions of the fly thorax, called the Hairy-wing (Hw) phenotype. Later, the *Hw* alleles were shown to correspond to a gain of function of *ac* or *sc* functions, when it was found that revertants of *Hw* were *ac* or *sc* mutations, and that *Hw* alleles showed overexpression of the *ac* and *sc* genes in normal or ectopic positions (Campuzano *et al.* 1986; Garcia Alonso and Garcia-Bellido 1986; Balcells *et al.* 1988). These alleles were enlightening in proposing an instructive "bristle promoting" function for the *ac* and *sc* genes. The cytological breakpoints leading to *ac*, *sc*, and *Hw* phenotypes were shown by the work of the Juan Modolell group to extend over ~100 kb of DNA (Campuzano *et al.* 1985). This implied that partial or noncomplementation between alleles of the same gene extended through huge distances of DNA!

DEVELOPMENTAL FUNCTION OF THE *achaete–scute* GENE COMPLEX

A genetic analysis of *ac* and *sc* mutations appeared in Genetics at about the same time as another article on the developmental genetics of the *ac–sc* system (Garcia-Bellido and Santamaria 1978; Garcia-Bellido 1979). The obvious question, at the time, was to know the phenotype of the total lack of function of the *ac* and *sc* genes including the *l'sc* region. This question was first addressed by Stern (1935), when he used a major deletion of the genes that also included the gene *yellow* (*y*) [the deficiency element of $T(1:2)sc^{19}$]. Stern looked for yellow chaetae in somatic recombination spots but found none, therefore concluding that $Df(1)sc^{19}$ was cell lethal. The analysis by Stern of *achaete* gynadromorphs, flies in which the male tissue was mutant for *achaete*, did show, however, that the mutation was acting in a cell-autonomous manner. Stern suggested that *achaete* was involved in "the response of cells to a predetermined invisible pattern" (Stern 1954, p. 240), the "prepattern," a concept that had a major influence in the understanding of bristle pattern formation (see below). We repeated the experiment labeling the $Df(1)sc^{19}$ cells with another cell marker, *forked* (f^{36a}) in addition to *y*, and found large spots in the notum devoid of chaetae, but composed of *forked* trichomes (Garcia-Bellido and Santamaria 1978). This result implied that $Df(1)sc^{19}$ cells have normal growth and viability, but failed to differentiate chaetae in the notum and in most of the adult cuticle. Cells homozygous for the $Df(1)sc^{19}$ deletion did, however, differentiate some chaetae in the wing margin—the mechanosensory chaetae of the triple row—suggesting the existence of yet another bristle-promoting gene located outside the region deleted by $Df(1)sc^{19}$. A subsequent analysis of a terminal deletion with a more proximal breakpoint [*Df(1)260-1*] concluded that this novel bristle promoting function, named *asense*, was also critical for the formation of a subset of sensory elements in the larval cuticle (Dambly-Chaudiere and Ghysen 1987). Clearly the *ac–sc* region included genes for several related functions and has since been called the *achaete–scute* complex (*AS-C*).

The best-known *AS-C* functions at the time were related to the formation of the adult peripheral nervous system (PNS), but the function of the *l'sc* region was still mysterious, because the smallest deletion including this gene [$Df(1)sc^{4L9R}$] was embryonic lethal. In gynandromorph mosaics, the $Df(1)sc^{4L9R}$ spots that appeared in the adult cuticle could form large territories that lacked only the chaetae missing in the $In(1)sc^{4}$ and $In(1)sc^{9}$ alleles (Figure 1B). In this manner, no adult phenotype distinct from that of the original inversions used to

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generate this deficiency could be associated with the deletion of the *l'sc* region. Interestingly, mutant territories that crossed the ventral midline killed the embryo, suggesting that the *l'sc* function was related to the formation of an essential ventral structure in the embryonic fate map, possibly the central nervous system (CNS) (Garcia-Bellido and Santamaria 1978). Subsequent work by the Campos-Ortega group confirmed the existence in the *l'sc* region of a function required for the formation of the CNS (Jimenez and Campos-Ortega 1979, 1990). We can now generalize by saying that the *AS-C* encodes four functions related to both PNS and CNS development in Drosophila. These functions promote the formation of sensory organs in the embryonic and adult peripheral neural systems and of neuroblasts in the central neural system. Accordingly, *AS-C* genes were named "proneural genes" (Ghysen and Dambly-Chaudiere 1988; Romani *et al.* 1989). The proneural genes were truly morphogenetic, as indicated by the existence of gain-of-function alleles causing supernumerary sensory organs. The developmental analysis of the *AS-C* also suggested that the same mutants affected the developmental pathways leading to the formation of chaetae and the CNS. The detailed analysis of cells deficient for the *AS-C* induced by mitotic recombination in the last stages of larval development indicated that the differential divisions of the chaetae mother cell occurred between 48 hr before puparium formation and 12 hr after puparium formation (APF) and that the macrochaetae completed their development earlier than the microchaetae. This analysis also showed that chaetae were able to develop in the absence of the *AS-C*, but only when its removal took place at the time of the differential divisions of the chaetae mother cell (Garcia-Bellido and Merriam 1971).

MOLECULAR ORGANIZATION OF THE *achaete–scute* GENES

Many questions related to the genetic organization and function of the *AS-C* had to wait for molecular analysis, which was undertaken by Juan Modolell and his colleagues during the 1980s and 1990s (Carramolino *et al.* 1982; Campuzano *et al.* 1985; Romani *et al.* 1987; Ruiz-Gomez and Modolell 1987; Villares and Cabrera 1987; Alonso and Cabrera 1988; Gonzalez *et al.* 1989; Dominguez and Campuzano 1993; Martin-Bermudo *et al.* 1993). The first challenges were to clone the genes, identify the coding regions, and molecularly map the existing breakpoints. This molecular information was critical to understanding how the large DNA extent of the *AS-C* related to the observed interactions between the sc-α, sc-β, and *asense* regions. The mapping of mutant alleles established the molecular limits of the *ac*, *sc*, *l'sc*, and *ase* genes and identified the transcripts corresponding to these genetic regions, which were called T5 (*ac*), T4 (*sc/sc*-α), T3 (*l'sc*), and T8/T1a (*ase*) (Figure 1B). The *sc*-β region contained the T2 transcript, but this was not related to a bristle promoting function.

The *sc* alleles mapped in ~40 kb of DNA 3′ of the T4 (*sc*) transcript, and their phenotypes were more severe the closer they were to this transcript. At the time, these mutants were thought to cause long-range structural perturbations leading to a reduction in *sc* transcription, which were stronger the closer they were to the transcript, which combined with a differential sensitivity of each macrochaeta to a given reduction in the amount of Ac/Sc ("threshold"). This scenario would result in the observed seriation of affected bristles (Campuzano *et al.* 1985). However, a subsequent analysis of ~70 X chromosome terminal deficiencies ending in the 5′ region of the T4 transcript uncovered a completely different order of sensitive bristles, although still the phenotypes depended on the distance from the gene of each deficiency endpoint (Ruiz-Gomez and Modolell 1987). To explain the existence of these two different 3′ and 5′ seriations, it was proposed that *sc* alleles disconnected flanking *cis*-regulatory elements from the transcript: the closer any breakpoint was from the T4 transcript, the stronger the phenotype because more *cis*-regulatory elements would be disconnected from the gene (Ruiz-Gomez and Modolell 1987).

Other work, reviewed in Ghysen and Dambly-Chaudiere (1988), Campuzano and Modolell (1992), and Gomez-Skarmeta *et al.* (2003), showed that the four AS-C proteins contain basic-HLH motifs previously found in the Myc oncogene, and that they regulate gene expression. The large noncoding DNA regions within the complex should then contain *cis*-regulatory regions ("enhancers") with sequences regulating the expression of the coding regions in specific epidermal positions and cell types. These enhancers are the key to understanding positional specificity and the peculiarities of step allelomorphism, because they act at a distance in *cis* to regulate the distantly located coding regions. Thus, breakpoints disconnect enhancer regions from the promoters, preventing *trans*-regulatory interactions, and lead to the absence of particular neuroblasts or sensory mother cells in the CNS and PNS, respectively. The discovery of enhancer sequences in the *AS-C* located at large distances from the coding regions was one of the first examples of the positional specificity of *cis*-regulatory DNA likely targeted by *trans*-regulatory proteins. This example was later shown to be a general aspect of eukaryotic gene organization and extends to many other gene complexes (such as the *bithorax* complex) where enhancer sequences were thought to correspond to specific genes (Lewis 1998). The question of positional specificity and temporal and spatial specification was thus transferred to the molecular recognition of *cis*-regulatory sequences by the products of *trans*-regulatory genes. These notions were

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fully developed in the emergent analysis of embryonic segmentation, when the hierarchy of maternal, gap, and pair-rule genes was dissected (Clyde *et al.* 2003). In this manner, the regulatory region of eukaryotic genes includes two types of sequences: one, inherited from prokaryotes that correspond to the "promoter" or "operator" region, and a second, one that can be very complex, exclusive of eukaryotes that can be called the "modulator" region containing the enhancer sequences. The first region is where the interaction with RNA polymerases and other multiprotein regulatory complexes occurs, whereas the modulator region defines when and where the gene will be transcribed through interactions with sequence-specific transcription factors.

EXPRESSION OF THE *AS-C* GENES

Trans-regulatory genes of the AS-C were searched for, using a "gene titration" approach. Like in bacteria, it was expected that an increase in the number of doses of the promoter could lead to a relative insufficiency of repressor *trans*-regulatory gene products. Following random mutagenesis in these potential genes, phenotypes of extrachaetae (similar to the *Hw*) were found in two loci, *extramacrochaetae* (*emc*) and *hairy* (*h*) (Botas *et al.* 1982). Both genes also encode HLH proteins that regulate the expression of *ac* and *sc* (Hairy) and that interact with the Ac and Sc proteins, antagonizing their function (Emc) (Ellis *et al.* 1990; Garrell and Modolell 1990; Van Doren *et al.* 1991, 1994; Ohsako *et al.* 1994; Campuzano 2001). Similar mutagenesis experiments using a mutant background heterozygous for *AS-C* deficiencies did not yield any candidate for an activator gene. However, these were later found using other approaches (see below).

The cloning of the *AS-C* genes allowed a number of experiments that deepened the understanding of the mechanisms leading to bristle patterning. First, the expression of the genes was described in detail in the wing imaginal disc—the epithelium that gives rise to the thorax and wing of the fly—in the embryonic peripheral and central nervous system and in several other tissues from which sensory organs developed (Cabrera *et al.* 1987; Romani *et al.* 1987, 1989; Cubas *et al.* 1991; Martin-Bermudo *et al.* 1991; Skeath and Carroll 1991, 1992; Dominguez and Campuzano 1993; Ruiz-Gomez and Ghysen 1993). The visualization of the expression patterns of the *AS-C* genes, by *in situ* hybridization first and then by using antibodies directed against the proteins, revealed a common scenario in which the genes were first expressed in groups of cells, the so-called "proneural clusters," and then accumulated at higher levels in the cell that enters the neural fate, the sensory mother cell (SMC) or the neuroblast (Figure 1C). The expression of the *AS-C* genes, the modifications to this pattern observed in *AS-C* mutants, and the use of additional cell markers specifically expressed in the neural precursors, together with the finding that AS-C proteins are transcription factors, marked a high point in the developmental analysis of chaeta formation and its relationships with the *AS-C* genes. In this manner, the complex pattern of sensory elements could be largely reduced to the generation of a landscape of proneural clusters where the *AS-C* genes were expressed. Similarly, the complex and puzzling complementation patterns among *sc* mutations came to be understood as a consequence of the existence of *cis*-regulatory regions directing gene expression in individual proneural clusters (Ghysen and Dambly-Chaudiere 1988; Campuzano and Modolell 1992; Modolell and Campuzano 1998). Interestingly, the same enhancers control the expression of *ac* and *sc*, and therefore both genes are expressed in the same pattern of proneural clusters (Ruiz-Gomez and Ghysen 1993; Gomez-Skarmeta *et al.* 1995). If the specific effects of *ac* and *sc* mutations involved only the enhancers they each affect, why do the two sets remove complementary subsets of bristles? This question could be only partially solved when point alleles in the *sc* (Gomez-Skarmeta *et al.* 1995) and *ac* (Marcellini *et al.* 2005) genes were characterized. Surprisingly, hemizygous males for a *sc* null allele (sc^{M6}) lost only a few micro- and some macrochaetae, whereas flies null for *ac* (ac^{cami}) were entirely normal (Figure 1, A and B). The *ac* and *sc* double mutant ($sc^{10.1}$) lacks all macro- and microchaetae (Figure 1, A and B), reinforcing the notion that the corresponding proteins have some degree of functional redundancy. These observations suggested that there are no qualitative position-specific differences between the Ac and Sc proteins with regard to their proneural function, although the mutant phenotypes of individual alleles and the study of *ac* and *sc* overexpression phenotypes indicated that the proneural activity of Sc is more effective than that of Ac (Rodriguez *et al.* 1990; Gomez-Skarmeta *et al.* 1995; Marcellini *et al.* 2005).

The positional specificity of the *sc* null mutant must be related in part to differences in the expression of other genes that somehow determine the probability of SMC formation for each amount and activity of proneural protein. These positional differences are likely to explain the puzzling observation that transient and generalized expression of Sc is able to direct bristle formation in the correct positions in homozygous *AS-C* mutant backgrounds ($sc^{10.1}$), *i.e.*, in wing discs lacking patterned expression of *AS-C* (Rodriguez *et al.* 1990). This inferred underlying layer of positional information may be conferred by the heterogeneous distribution of Emc, which affects the activity of AS-C proteins (Cubas and Modolell 1992), and by the heterogeneous expression of other genes involved in "lateral inhibition" (Vassin *et al.* 1987; de Celis and Garcia-Bellido 1994; Parks *et al.* 1997; Joshi *et al.* 2006). In this manner, it appears that bristle positions are determined both by

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the restricted expression of *AS-C* genes, controlled by modular enhancers, and by the heterogeneous expression of other genes that modulate the proneural activity of the Ac/Sc proteins or locally modify the response of the tissue to the neuralizing effects of these proteins.

Several related questions, not yet satisfactorily solved, have emerged from the description of *AS-C* expression and the dynamics of SMC appearance and differentiation. The first question relates to the nature of the elusive positive regulators responsible for the activation of *AS-C* expression in each proneural cluster. The second issue concerns the mechanisms of SMC selection among the cells expressing *AS-C* in the proneural cluster. Finally, the actual role of the AS-C proteins in conferring neural potential remains mysterious, in part because the genes *ac* and *sc* are no longer expressed once the SMC starts its differential divisions (Cubas *et al.* 1991); only *ase* expression persists in the SMC (Dominguez and Campuzano 1993).

Cis-REGULATION OF THE *AS-C*

The understanding of the regulation of *AS-C* expression followed the complementary approaches of (1) dissecting each regulatory region (the "position-specific enhancers") by making fusion constructs with a reporter gene and (2) searching for mutants affecting the formation of specific subsets of macrochaetae. The first mutation identified as a candidate to participate in the position-specific activation of the *AS-C* in the thorax was named *iroquois* (*iro*), because it caused a phenotype in which several of the lateral macrochaetae were missing (Dambly-Chaudière and Leyns 1992; Leyns *et al.* 1996). The genetic and molecular analysis of *iro* uncovered yet another gene complex, the *iro-C*, formed by three genes, *caupolican*, *araucan*, and *mirror* (Gomez-Skarmeta *et al.* 1996; McNeill *et al.* 1997; Kehl *et al.* 1998). These genes encoded related nuclear proteins characterized by the presence of a conserved homeodomain. They have different functions depending of the developmental context, and during the appearance of the proneural clusters they are expressed in a pattern that partially overlaps some of the clusters (Cavodeassi *et al.* 2001). The mechanism of action of the Iro proteins is still unknown, and although they are required for the correct expression of the *AS-C* genes in the most lateral proneural clusters, they seem to act as transcriptional repressors (Cavodeassi *et al.* 2001; Bilioni *et al.* 2005). Other candidate transcriptional activators of *AS-C* expression were also identified by virtue of their restricted expression pattern in the thorax and their effects on specific macrochaetae. For example, the GATA-containing protein Pannier (Pnr) is expressed in the region from which the dorsocentral macrochaetae form and directly regulates the expression of *ac/sc* by binding to a specific *AS-C* enhancer (Garcia-Garcia *et al.* 1999). Similarly, the Zn-finger proteins Spalt and Spalt-related are also expressed in specific domains of the thorax and are required for the formation of the anterior notopleural macrochaetae (de Celis *et al.* 1999). The identification of *ac/sc* activators in the thorax suggests that pattern formation in this tissue is the consequence of a progressive deployment of transcription factors whose expression is restricted to specific territories. Therefore, the epithelium contains a landscape of transcription factors acting in a combinatorial manner to confer a genetic identity on each region of the thorax. This landscape has been referred to as the prepattern, following the classic definition that Stern used to explain the competence to develop bristles in genetic mosaics bearing *ac* mutant clones (Stern 1954; Ghysen and Dambly-Chaudiere 1988; Campuzano and Modolell 1992). In this scenario, the AS-C *cis*-regulatory regions work as a decoding device that reads out different combinations of transcriptional regulators, the prepattern proteins, and converts them into ON and OFF states of transcription for both *ac* and *sc*, resulting in the formation of individual proneural clusters (Gomez-Skarmeta *et al.* 2003). Much work is still needed to understand the dynamics of proneural cluster formation and extinction and the manner in which they then relate to the singling out of individual cells in constant positions. This is the final issue addressed in this overview.

CELL INTERACTIONS WITHIN PRONEURAL CLUSTERS

The transition from proneural clusters to individual SMCs became a paradigmatic example of a patterning mechanism that refines cell commitment from groups of cells to individual cells. Two classic observations relate to this mechanism. First, in the pioneering description of the development of *Notch* mutant embryos by Poulson in the 1940s, it was reported that an excess of neural tissue in *Notch* mutant embryos developed at the expense of the epidermis (Poulson 1940). This observation suggested that ventral ectodermal cells have the potential to develop as neural elements and that Notch activity was somehow involved in the repression of this fate, therefore allowing the formation of epidermal cells. Thus in the *Notch* mutant embryo all ventral ectodermal cells follow what was understood to be the primary fate, *i.e.*, neural development. Curt Stern, who analyzed the behavior of *ac* mosaics in the thorax, made the second key observation: when *ac* mutant tissue includes the position of the anterior or the posterior dorsocentral bristles, they fail to differentiate (see above), but instead, in a number of cases, a nonmutant macrochaeta appears close to, but not in, the wild-type position (Stern 1954). This indicated that several cells near the position of a normal macrochaeta are competent to form a bristle, and that in a normal situation the formation of one bristle in this field prevented other

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cells from accomplishing the same fate. These observations were followed by cell ablation experiments, carried out in grasshopper embryos, which indicated that the epidermal cells in the vicinity of a developing neuroblast entered the neural pathway when this neuroblast was killed (Taghert *et al.* 1984). Several authors realized that the Notch phenotype and the mechanism of cell fate inhibition by the SMC or neuroblast (lateral inhibition) were related phenomena (Knust and Campos-Ortega 1989). Furthermore, the mutagenesis screens carried out by Nusslein-Volhard and Wieschaus (1980) identified additional genes with hyperplasic CNS (the "neurogenic" phenotype) (Campos-Ortega and Knust 1990). This opened the possibility of connecting the function of a group of genes (the neurogenic genes as they were called) with the cellular mechanism of lateral inhibition (Knust and Campos-Ortega 1989; Campos-Ortega 1993). Today we know that the neurogenic genes encode members of a universally conserved signal transduction pathway, the Notch signaling pathway, that during SMC singling out prevents the accumulation of proneural protein in a cell by interfering with a loop of *ac* and *sc* self-stimulation mediated by SMC-specific enhancers present in the *AS-C* (Culí and Modolell 1998; Giagtzoglou *et al.* 2003). Thus, the Ac/Sc proteins in the proneural cluster cells promote activation of Delta, the ligand of the pathway, which in turn activates the Notch receptor in neighboring cells. This impairs the activity of the SMC-specific enhancers and maintains most of the cells of a proneural cluster in a non-SMC state ("mutual inhibition"). The cell with the highest levels of Ac and Sc escapes from the inhibition, activates the SMC enhancers, accumulates maximal levels of Ac and Sc, and becomes the SMC. The SMC then signals most strongly to the remaining cells of the cluster and prevents them from becoming additional SMCs (lateral inhibition). In summary, by linking Ac/Sc expression in proneural clusters to Delta and Notch signaling, and this to repression of the SMC-specific enhancers, differences in proneural gene activity lead to the selection of single SMCs. The detailed molecular analysis of the regulatory relationships between the Notch signaling pathway and the proneural genes is still a work in progress (Jennings *et al.* 1994; Giagtzoglou *et al.* 2003; Castro *et al.* 2005; Acar *et al.* 2006; Pi and Chien 2007).

BEYOND NEUROGENESIS: OTHER ROLES OF THE AS-C GENES

The AS-C was perhaps the first example of a gene or group of genes positively linked to a key developmental decision, that of forming a neural precursor, and consequently the *AS-C* has been mostly studied in the context of neurogenesis. Surprisingly, a more exhaustive analysis of its expression pattern and phenotype uncovered several functions not related to neural development. For example, the *AS-C* genes are expressed in clusters of mesodermal cells, from which muscle progenitors form through a mechanism of lateral inhibition mediated by Notch signaling (Bate *et al.* 1993; Carmena *et al.* 1995). In this system, the loss of *AS-C* function leads to the absence of individual muscle progenitors. Similarly, the *AS-C* genes are also required for cell fate assignment of specific cells in the gut, a tissue of endodermal origin (Tepass and Hartenstein 1995). In this manner, a key invariant aspect of the *AS-C* genes is their participation in the selection of committed cells from groups of competent cells, through processes of lateral inhibition. One can speculate that the connection between the AS-C and the Notch pathway is phylogenetically old and has been retained during evolution and adapted to a variety of developmental contexts as a device ensuring single-cell resolution in cell-fate allocation.

Since the identification of the AS-C proteins as transcription factors bearing a bHLH domain, many orthologs have been identified and characterized within the framework of the developmental mechanisms of sensory organ pattern formation (Bertrand *et al.* 2002; Sugimori *et al.* 2007). The proteins belonging to the AS-C family identified in other invertebrate and vertebrate genomes share functional features with the fly orthologs. Thus most vertebrate *AS-C* genes are expressed principally in the developing nervous system, where they participate in the selection of neural progenitor cells and mostly in the differentiation of specific neuronal lineages. As happens in the fly, the expression of vertebrate proneural genes is turned off before the progenitor cell begins to differentiate, suggesting that a key aspect of their function is to initiate a cascade of transcriptional regulation leading to sequential steps of cell determination and differentiation (Bertrand *et al.* 2002; Chang *et al.* 2008).

CONCLUSION

The analysis of the *AS-C* uncovered several trends common to many developmental processes and provided a framework to dissect the molecular bases of pattern formation, regional specification, and cell commitment. It is a very illustrative example of the difficulties of applying genetic analysis to complex genes, because many sound and internally consistent proposals could be contrasted and accounted for only after the cloning and molecular study of the genes. Apart from telling the story of the analysis of chaetae pattern formation, the *AS-C* has been instrumental in understanding the organization of eukaryotic genes, with their complex arrays of *cis*-regulatory modules influencing the expression of adjacent transcription units, and was also a key entry point for the analysis of the genetic subdivisions of developmental territories by partially overlapping domains of gene expression (prepattern). Finally, the identification of the *AS-C* genes as

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proneural also helped to identify their vertebrate counterparts and to begin to understand the molecular mechanisms of neural cell-type specification.

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