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The genetics of the *Drosophila achaete-scute* gene complex: a historical appraisal

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ABSTRACT The *Drosophila achaete-scute* complex consists of four genes encoding transcription factors of the bHLH family. Due to their intricate organization, these genes have occupied geneticists and developmental biologists for many years. Here, genetic studies on the complex are discussed from a historical point of view.

KEY WORDS: *achaete-scute complex, Drosophila, organization*

Introduction

Studies on the organization and function of the *Drosophila achaete-scute* complex (AS-C), a cluster of four genes at the distal tip of the X-chromosome, constitute a long and interesting chapter in the history of *Drosophila* genetics. It is therefore not surprising that the AS-C has been the subject of a multitude of reviews and other essays on either its genetic organization or, more frequently, its developmental function. And yet, this is still a very appropriate topic for consideration in a *Festschrift* dedicated to Antonio García-Bellido, for he has made a major contribution to the study of the AS-C. My intention in writing what follows was to discuss studies on the AS-C more or less chronologically, listing the major problems with which the gene complex has confronted investigators and the discoveries that contributed to solving them. I concentrate on the genetic studies, where I see a major source of confusion and paradox, and am therefore forced to neglect to some extent discussion of the developmental functions of the AS-C.

Mutations of the AS-C genes are generally viable and elicit conspicuous phenotypes in the adult flies, characterized either by a lack of particular sensory organs (*achaete* and *scute* mutations), or the development of supernumerary ones (*Hairy wing*, *Hw*, mutations). *scute* mutations affect specific sets of macrochaetes, or large bristles. Particularly striking are those on the scutum in the mesothorax of the fly, which is the reason for the name given to the mutations. *achaete* mutations affect some macrochaetes, but also the smaller microchaetes. *Hw* mutations, which cause the opposite phenotype, were found to map to the same locus. It was soon recognized that *achaete* and *scute* mutations uncover two different genes, since, besides having different phenotypes, they usually complement each other. Nevertheless, the genetic analysis of *achaete* and *scute* mutations has, since their discovery, presented unusually difficult problems.

Allele specific phenotypes: is the gene divisible?

The genetic analysis of these mutations began in the late 1920s when the Russian geneticist Serebrovsky and his colleagues started to irradiate flies to study the relationships between the chromosomal theory of heredity and the presence-absence hypothesis, postulated by Bateson. Bateson had introduced mnemonic symbols to refer to alleles, for example, *Y* and *y* for yellow and green seeds, and proposed that recessive alleles (as *y*) corresponded to the absence of the dominant allele *(Y)*. The existence of series of alleles of the same gene with different expressivity was a strong argument against the latter hypothesis. The idea put forward by Serebrovsky was that both theories could be reconciled if the postulate of the indivisibility of the gene were abandoned (see Carlson, 1966): the gene could be composed of a group of subgenes, each of which fulfils part of a specific function. Allemomorphs with different expressivity could then be explained by assuming that the mutational process affected different subgenes of the same group.

Dubinin recovered mutations which exhibited a striking phenotypic specificity, in that each suppressed the development of a defined group of bristles. In crosses of flies carrying different alleles that affected partially overlapping sets of macrochaetes, partial complementation was found: the heterozygotes differentiated the allele-specific bristles, but not those bristles affected by both parental alleles. Alleles were also found that affected non-overlapping groups of bristles. If flies carrying alleles of the latter type were crossed, the resulting transheterozygotes were wild type in phenotype. Such alleles were called step-allelomorphs. To explain this peculiar behavior, Dubinin assumed that the *scute* gene was composed of a number of subgenes, each controlling the development of a specific bristle or group of bristles. He also proposed that *achaete* and *scute* might share some of the subgenes

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that each was assumed to contain. Therefore, in this hypothesis the gene was divisible and the territory of two contiguous genes overlapped such that their limits could not be defined with precision (Carlson, 1966). While (still disputable) arguments against the latter conclusion were raised shortly thereafter, an explanation for the apparent divisibility of the gene did not emerge for a much longer time.

The "left-right" test

The hypothesis that genes overlap in their chromosomal territories was disproved by Muller using his "left-right" test (Muller, 1935). He collected several inversions with *achaete-scute* phenotypes and made the stocks isogenic to eliminate modifiers (Muller, 1935,1955; Muller and Prokofjeva, 1935; Raffel and Muller, 1940). Then he crossed males carrying one of the inversion chromosomes with females carrying a different one. If the distal breaks were at different positions in the *achaete-scute* region, one of the two products of meiotic recombination events occurring anywhere in the inverted part of the X-chromosome would carry the left segment of one X-chromosome and the right portion of the other, and thus be deficient for the region of the *scute* locus between the two inversion breaks. Animals carrying this deficiency chromosome should consequently show an extreme phenotype. In contrast, the other recombination product would carry the right segment of the first X-chromosome and the left portion of the other one, and therefore be duplicated for the same region. Animals with this X-chromosome were expected to be phenotypically wildtype. If the breakpoints of the two inversions tested were in the same region of the *scute* locus, the two recombination products would be identical.

With this technique, Muller defined four different regions of the *scute* locus at which the X-chromosome had been broken by irradiation. These regions delimited three genes with distinct, invariable phenotypes, which corresponded to *achaete*, *scute* and a new gene that Muller called *lethal of scute* (*l'sc*). No overlap between adjacent genes was observed. In addition, Raffel and Muller (1940) concluded that their study did not provide compelling evidence for divisibility of the gene. However, they could not completely reject this possibility either.

Developmental genetics of the AS-C: the sensilla and the central nervous system

To analyze the development of the pattern of bristles, Antonio García-Bellido started work on the genetic organization of the AS-C in the mid-seventies. In a paper published in 1979, several important points were raised. First, he reported having repeated the analysis of left-right recombinants, thus confirming Muller's results and conclusions. In addition to the recombinants, however, García-Bellido used newly constructed deficiencies and duplications in order to define what he called "phenotypic breakpoints". By combining deficiencies and duplications, he created other small synthetic deletions in the entire region and established correlations between given breakpoints and phenotypes. García-Bellido defined a maximum of eight different breakpoints associated with *scute* phenotypes, located on each side of the *l'sc* gene. On the basis of this observation, he proposed that the "…*achaete-scute* system appears as a tandem reverse repeat of similar functions at both sides of *l'sc*.…". Thus, a *scuteβ* function located proximal to *l'sc* was distinguished from a *scuteα* function, located distally (see Fig. 1). However, additional *scute* functions were assumed to exist. He also emphasized the lack of qualitative differences between the phenotypes of rearrangements located on each side of *l'sc*, suggesting that "…the *scute* functions affected by the rearrangements appear to be redundant." Moreover, he reported that *Hw* phenotypes can be generated by internal duplications of the AS-C. Granted that these phenotypes can also be produced by mutations in *hairy* or *extramacrochaete* (*emc*, see below), two second-site suppressors of the AS-C, García-Bellido (1981) proposed that *Hw* corresponds to an excess of AS-C gene function (see García-Alonso and García-Bellido, 1988). Most of these hypotheses and conclusions have since been confirmed.

García-Bellido and Santamaria (1978) studied the developmental effects of some of the chromosomal aberrations used in the genetic analysis on genetic mosaics, i.e., in clones generated by mitotic recombination events and in gynandromorphs. They concluded that *achaete* and *scute* are required for the differentiation of adult sensory organs, and proposed an involvement of *l'sc* in the development of the CNS. This latter proposal was based on the observation that gynandromorphs died whenever male tissue mutant for *l'sc* extended into the embryonic primordium of the CNS. Histological support for this proposal, and confirmation of the observed functional redundancy of the AS-C genes, was obtained soon thereafter, when embryos carrying deletions of various sizes were studied (Jiménez and Campos-Ortega, 1979,1987; Campos-Ortega and Jiménez, 1980; White, 1980). García-Bellido (1979) had observed that the severity of the bristle defects caused by deletions depended on the size of the deletion, being "…more extreme phenotypically, the more separated are the presumed breakpoints." A similar conclusion was drawn with respect to CNS development: *l'sc* was found to be essential, but the severity of the CNS lesions increased when other genes were also deleted. Those genes included *achaete* and *scute*, as well as genes further proximal to *l'sc*, i.e., the *scuteβ* locus, *ventral nervous system condensation defective* (*vnd*, White, 1980; White *et al.,* 1983), and another, previously unidentified, gene between *scuteβ* and *vnd*, which following a suggestion of Ghysen and Dambly-Chaudière, who had made similar observations with respect to the sensory organs (see below), was called *scuteγ* (Jiménez and Campos-Ortega, 1987).

Campos-Ortega and Jiménez (1980) and Dambly-Chaudière and Ghysen (1987) showed that the AS-C is also required for development of larval sensory organs. Dambly-Chaudière and Ghysen (1987) discovered that this requirement is restricted to the external sensory organs, while the chordotonal and other internal sensory organs are not affected by deletions that eliminate all of the AS-C. Ghysen and Dambly-Chaudière (1988) eventually renamed *scuteγ*, the genetic function further proximal to *scuteβ* required for CNS and sensory organ development, as *asense* (González *et al.,* 1989).

Molecular organization of the AS-C: four transcription factors of the bHLH family

The genomic DNA of the AS-C region was cloned and partially characterized in 1982 (Carramolino *et al.,* 1982; see Fig. 1). The breakpoints of several rearrangements associated with *achaete*

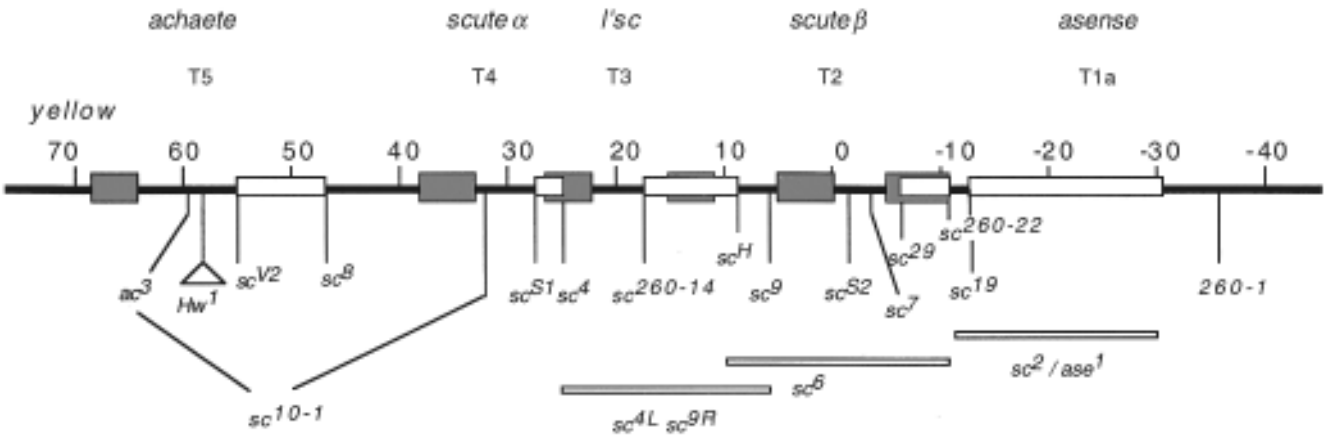

Fig. 1. Schematic representation of the organization of the AS-C. *Compiling data from Campuzano et al., 1985, Gómez-Skarmeta et al., 1995, and Parras et al., 1996. Map units are given in kb (numbering according to Carramolino et al., 1982). At the top, the genetic functions and the corresponding T transcripts are indicated. The centromere is to the right. The stippled boxes on the molecular map indicate the fragments in which cis-regulatory sequences of T4 and T5 are located (Gómez-Skarmeta et al., 1995). The white boxes on the map indicate the extent of deletions having an effect on T3 expression, thus implying regulatory sequences of T3 (Parras et al., 1996). The vertical lines indicate the position of breakpoints and other mutations, the white bars below the extent of three different deletions, two of them mentioned in the text. Notice the large extent of the region to which mutant phenotypes have been mapped.*

and *scute* mutations were mapped molecularly, allowing a preliminary definition of the limits of *achaete*, *scuteα*, *l'sc* and *scuteβ*. Six transcripts, called T1 to T6, were initially identified within the cloned region, and a correlation between transcripts and some of the breakpoints was established (Campuzano *et al.,* 1985). On this basis it was proposed that T5 corresponds to *achaete*, T4 to *scuteα* and T3 to *l'sc*. The discovery that all three encode transcription factors of the bHLH family (Villares and Cabrera, 1987; Alonso and Cabrera, 1988; Martin-Bermudo *et al.,* 1993), added considerable strength to this contention. *asense*, the *scuteγ* function identified on the basis of synthetic deletions further proximal to *scuteβ* (Dambly-Chaudière and Ghysen 1987; Jiménez and Campos-Ortega, 1987), was proposed to correspond to a transcript also encoding a bHLH protein, called T8 (Alonso and Cabrera, 1988) or T1a (González *et al.,* 1989). The molecular analysis of several *Hw* mutations also provided convincing evidence that the *Hw* phenotypes are due to overexpression of the *achaete* and *scute* genes (Campuzano *et al.,* 1986; García-Alonso and García-Bellido, 1986; Balcells *et al.,* 1988). T6 was identified as *yellow* (Campuzano *et al.,* 1985; Chia *et al.,* 1986). T2 is expressed in the midgut and encodes an aspartic acid protease (F. González and S. Romain, personal communication), and thus appears to be unrelated to the functions of the AS-C. Hence, the molecular nature of *scuteβ* remained still an open question.

"Phenotypic breakpoints" at the molecular level

Two striking, and most confusing, features of the AS-C are that *scute* phenotypes (i) are associated with two separate loci, *scuteα* and *scuteβ*, and (ii) that they can be ordered in a series according to their severity, i.e., the number and position of affected sensilla (García-Bellido, 1979). Indeed, the preliminary molecular data on several rearrangements associated with *scute* mutations confirmed that the two *scute* loci, *scuteα* and *scuteβ*, defined genetically, flank the T3 *(l'sc)* transcription unit (Campuzano *et al.,* 1985). Ruiz Gómez and Modolell (1987) mapped the breakpoints of 74 terminal deficiencies (Mason *et al.,* 1984,1986) within the cloned genomic DNA and accurately defined "phenotypic breakpoints" (García-Bellido, 1979) associated with loss of specific bristles, at the molecular level. A similar analysis was carried out by Leyns *et al.* (1989) with respect to the pattern of campaniform sensilla on the wing blade, using the same terminal deficiencies and an *achaete*$^{+}$ duplication in order to restrict the analysis to *scute*. The main conclusion of both papers was that *scuteα* comprises a number of functional units, representing cis-regulatory sequences, each directing gene activity in a given sensory organ. These observations also provided evidence to explain the seriation of *scute* phenotypes (García-Bellido, 1979) as the result of impairment of increasing numbers of regulatory sequences in the rearrangements. Moreover, the analysis of the *scute*^{6} mutation, an internal deletion of about 20 kb proximal to the T3 transcription unit, suggested that additional *scute* regulatory sequences in this region account for the *scuteβ* phenotypes (Ruiz Gómez and Modolell, 1987).

Proneural clusters

The analysis of the spatial pattern of expression of the AS-C transcripts had important conceptual consequences (Cabrera *et al.,* 1987; Romani *et al.,* 1987,1989; Cubas *et al.*, 1991; Martin-Bermudo *et al.*, 1991; Skeath and Carroll, 1991,1992; Ruiz-Gómez and Ghysen, 1993). Three of the transcripts (T3, T4 and T5) were found to be initially expressed in cell clusters within both the embryonic neuroectoderm and the imaginal discs; a single cell in each cluster differentiates as a neural progenitor, the remainder giving eventually rise to epidermal cells. AS-C genes were called then proneural genes (Ghysen and Dambly-Chaudière, 1989; Romani *et al.*, 1989), for they promote neural development: in AS-C mutants the corresponding progenitor cells are missing (Romani *et al.,* 1989; Jiménez and Campos-Ortega, 1990; Ruiz-Gómez and Ghysen, 1993); the realms of transcription of T3, T4 and T5 were

called proneural clusters (Simpson, 1990). One striking finding was that T4 and T5 are transcribed in essentially identical patterns, i.e., within the same cells, in the embryonic neuroectoderm and the imaginal proneural clusters. How this expression pattern is regulated remains an intriguing feature.

The expression profile of the *asense* transcript is rather different: T1a (or T8) is not expressed in proneural clusters, but in the neural progenitor cells and their progeny (Alonso and Cabrera, 1988; González *et al.,* 1989; Brand *et al.,* 1993; Dominguez and Campuzano, 1993). On this basis, Brand *et al.* (1993) have called *asense* a neural precursor gene to distinguish it from the other members of the AS-C, or genuine proneural genes. However, *asense* has a proneural function for the development of chemosensory bristles at the anterior wing margin (Dominguez and Campuzano, 1993).

Within proneural clusters, selection of neural progenitor cells is mediated by the neurogenic genes (Brand and Campos-Ortega, 1988; Cabrera, 1990; Skeath and Carroll, 1992; Martin-Bermudo *et al.,* 1993; Ruiz-Gómez and Ghysen, 1993), a set of genes, loss-of-whose-function causes misrouting into neurogenesis of all the proneural cells (Lehmann *et al.,* 1981,1983; Hartenstein and Campos-Ortega, 1986; Simpson and Carteret, 1989; Goriely *et al.,* 1991; Campos-Ortega and Haenlin, 1992). The genes of the E(SPL)-C can now be defined as the major trans-regulators of the AS-C genes during development of the proneural clusters (Brand and Campos-Ortega, 1988; de Celis *et al.,* 1991; Tata and Hartley, 1995; Nakao and Campos-Ortega, 1996). Much has been written on the relationships between proneural and neurogenic genes and the analysis of the regulatory network formed by the products of all these genes is still subject of intense research. Since this is not my primary concern here, I will not elaborate further on this topic.

The cis-regulation of the AS-C: genetic functions and transcripts

The molecular mapping of mutations, i.e., the determination of the phenotypic breakpoints, and the identification of the AS-C transcripts seemed to have clarified the genetic organization of the AS-C -the most complex and controversial aspect of the story. Four transcripts encoding transcription factors of the bHLH family were proposed to correspond to four functions defined genetically: *achaete*, *scute*, *l'sc* and *asense*; the *scuteβ* locus appeared to consist of nothing else but cis-regulatory sequences of *scute*. However, as the cis-regulation of transcript expression was studied in more detail, weak points appeared in this argument (Fig. 1).

The spatial patterns of T5 and T4 expression are very similar, if not identical, both in the embryo and the imaginal discs (Cabrera *et al.,* 1987; Romani *et al.,* 1989; Cubas *et al.,* 1991; Skeath and Carroll, 1991). However, the complexity of cis-controlling sequences appeared to be much lower for T5 than for T4 (Ruiz-Gómez and Modolell, 1987). How can the patterns of the two transcripts be so similar, if the requirements for their regulation are so different? In order to explain coexpression of T5 and T4, cross-regulation between the two genes has been invoked. Both transcripts were thought to be activated in complementary spatial domains early in development, but thereafter each gene product was thought to stimulate expression of the other in the proneural clusters. This possibility was supported by the ability of either T4 or T5 to stimulate reporter gene constructs reciprocally (Martinez and Modolell, 1991; Skeath and Carroll, 1991; Van Doren *et al.,* 1992; Martinez *et al.,* 1993).

However, several data indicate that there is not much cross-activation, but that both transcripts share regulatory enhancer elements active in the proneural clusters of the embryonic neuroectoderm (Skeath *et al.,* 1992; Ruiz-Gómez and Ghysen, 1993) and the wing imaginal disc (Gomez-Skarmeta *et al.,* 1995). Thus, in discs bearing *In(1)sc*^{8}, an inversion that breaks between T5 and T4 thus separating the two transcription units and regulatory sequences, coexpression is perturbed; in the *scute*^{M6} flies, in which no functional T4 protein is made, T5 protein accumulates in all proneural clusters as in the wild-type. The cis-regulation of the pattern of transcription is currently envisaged as the result of single regulatory elements that accurately direct expression of both T4 and T5 in the same regions of the neuroectoderm and imaginal discs. The functional significance of the coexpression of T4 and T5 in proneural clusters is unclear. It has been proposed that the amount of protein provided by one transcription unit alone is insufficient to drive development of the corresponding neural progenitors (Modolell, 1996). *achaete* and *scute* mutations differ in that the former affect microchaetes, as well as some macrochaetes, whereas the latter affect macrochaetes only. Hence, as yet unidentified enhancers are most probably located within the *achaete* region of the locus, which may be specific for driving development of microchaetes.

Preliminary data on the regulation of T3 expression in the neuroectodermal proneural clusters suggest a similarly complex situation (Fig. 1; Parras *et al*., 1996). The analysis of T3 expression in several mutants has permitted definition of five controlling regions of T3 scattered through 80 kb of genomic DNA (Martin-Bermudo *et al.,* 1993). To what extent T3 is regulated from some of the cis-regulatory elements of T5 and T4 is unknown.

Shared regulatory sequences pose the question as to how specific the encoded proteins are. Strictly speaking, the correspondence between transcripts and genetic functions is not yet proven. Unfortunately, rescue and overexpression experiments with these transcripts [see Rodriguez *et al.,* 1990, for T4 *(scute)*; Martin-Bermudo *et al.,* 1993, Hinz *et al.,*1994, and Giebel *et al.,* 1997, for T3 *(l'sc)*; and Brand *et al.,* 1993, and Dominguez and Campuzano, 1993, for T1a *(asense)*], have not provided conclusive support for the correlation of individual transcripts with specific genetic functions. Due to functional redundancy, ectopic expression of any of the three transcripts induces the production of the same sets of sensilla in the imaginal disc derivatives. For example, even though *l'sc* is dispensable for sensory organ development (García-Bellido, 1979; Dambly-Chaudière and Ghysen, 1987), and T3 is not transcribed in the wing disc, it can substitute for the loss of T4 and T5 if ectopically expressed in the wing disc (Brand *et al.,* 1993; Hinz *et al.,* 1994). A 3.2 kb DNA including the T3 transcription unit rescues the lethality and the CNS lesions associated with a deletion of the *l'sc* function (Martin-Bermudo *et al.,* 1993). However, in order to distinguish between a specific function of T3 and unspecific effects of redundant proteins, the same rescue experiment should be done with T4, T5 and T1a. With respect to the *scute* function, evidence in favor of its correspondence with the T4 transcript is provided by the s*cute*^{M6} mutation, in which a stop codon interrupts translation at the start of the HLH domain without affecting cis-regulatory regions. In these animals, two macrochaetes are suppressed in spite of the presence of a functional T5 protein

(Gómez-Skarmeta *et al.,* 1995), suggesting that the T4 protein provides sufficient specificity to permit development of at least these two particular macrochaetes. However, although the amounts of T4 and T5 protein have been reported to be similar in most proneural clusters, a slightly higher concentration of T4 protein is present in a few of them, including those affected in the *scute*^{M6} mutation (Gómez-Skarmeta *et al.,* 1995). Therefore, this observation alone does not allow one to decide between a specific function of T4 and differential dose requirements in the corresponding proneural clusters.

But not all is redundancy in the AS-C; a few functional differences between the proneural proteins have already been reported. Thus, whereas in mutants lacking the AS-C the proteins encoded by T5 and T4 fully rescue the lineage of the MP2 neuroblast, in which both are expressed, neither one of the other proneural proteins can (Parras *et al.,* 1996). Additional differences between T4, on the one hand, and T5 and T3, on the other hand, have also been described with respect to sex determination (Parkhurst *et al.,* 1990). Therefore, although conclusive evidence for specific functions of the proneural proteins in the clusters is still lacking, it seems reasonable to continue using the proposed correspondence between transcription units and genetic functions. Direct evidence for specific roles of the proteins under discussion will most probably be obtained soon.

The trans-regulation of the AS-C: pattern formation at the cellular level

The expression of the AS-C genes in proneural clusters, situated in well defined locations within the neuroectoderm and the imaginal discs, is dependent on the function of genes that act on cis-regulatory sequences in the promotor regions of the AS-C genes. However, the trans-regulation of the AS-C genes is still poorly understood.

Studies on the trans-regulation of the AS-C began when García-Bellido and coworkers (Botas *et al.,* 1982) described the results of what they called "gene-dose titration analysis". The rationale for such an analysis was that when two genes interact functionally, such that one regulates the other, changes in gene dosage, e.g., from the normal 2:2 to 1:3 or 1:4, may cause phenotypic changes. This work led to the discovery of the *extramacrochaete (emc)* gene and the recovery of new *hairy* alleles, as putative regulators of *scute*. These results, together with further studies by Moscoso del Prado and García-Bellido (1984), provided evidence that both *hairy* and *emc* are negative regulators of the AS-C. The available evidence shows that EMC and the proteins encoded by the E(SPL)-C act within the proneural clusters, *hairy* appears to be a general repressor of AS-C expression (see molecular data in Cubas and Modolell, 1992; Van Doren *et al.,* 1994; Dawson *et al.,* 1995; Fisher *et al.,* 1996).

Martin-Bermudo *et al.* (1991) and Skeath *et al.* (1992) showed that the position of the clusters of T3, T4 and T5 transcripts in the neuroectoderm is controlled by the genes that regulate pattern formation in the embryo: pair-rule and segment polarity genes control the establishment and maintenance, respectively, of the pattern along the antero-posterior axis, and *twist* and *snail*, *decapentaplegic* and *tolloid* with respect to the dorso-ventral axis. In a search for modifiers of AS-C genes, using a protocol similar to that of Botas *et al.* (1982), Dambly-Chaudière and Leyns (1992) found the *iroquois* locus (Leyns *et al.,* 1996). This locus comprises two different homeobox genes, *araucan* and *caupolican*, which act as trans-regulators to control the generation of the pattern of proneural clusters in imaginal discs (Gómez-Skarmeta *et al.,* 1996; Leyns *et al.,* 1996). Another putative trans-regulator of AS-C expression is *pannier* (Jürgens *et al.,* 1984), which encodes a zinc-finger protein required for the spatial regulation of *achaete* and *scute* in proneural clusters (Ramain *et al.,* 1993). Data on the embryonic expression of *pannier* are compatible with the assumption that the encoded protein suppresses proneural gene activity outside the neuroectoderm (Winick *et al.,* 1993). Finally, *vnd*, which encodes a homeodomain protein (Jiménez *et al.,* 1995), is an additional trans-regulator of the AS-C genes, at least within the medial regions of the embryonic neuroectoderm (Skeath *et al.,* 1994; Jiménez *et al.,* 1995).

The AS-C, *a posteriori*

The path followed to elucidate the genetic organization of the AS-C has been a rather tortuous one. In retrospect, four factors explain the difficulties encountered in the genetic analysis. First, the overwhelming majority of available *scute* mutations are rearrangements, e.g., inversions, insertions of transposible elements and translocations; a few are deletions and, to my knowledge, only one (*scute*^{M6}) is a true point mutation in the coding sequence of T4 affecting neither regulatory sequences nor other transcription units. Consequently, many mutations are hypomorphs and some of them, in addition, exhibit a variety of positional effects. This together with, second, the marked susceptibility of *achaete-scute* mutations to genetic and enviromental modifiers determined, in non-isogenic strains, some variability in phenotypic expression. Third, due to its location at the distal tip of the X-chromosome, meiotic recombination in the region of the AS-C is very low, making meiotic mapping of alleles extremely difficult. Most of these problems ultimately found satisfactory solutions when genetic and molecular approaches were combined. The use of gene deletions and isogenic strains avoids most of the problems caused by the study of hypomorphs; the precise mapping of breakpoints and regulatory sequences using molecular methods eventually permitted accurate correlation between genomic DNA and phenotypic defects.

The most important source of confusion has been, and remains, the organization of cis-regulatory sequences. On the one hand, sequences required for regulation of T4 and T5 are distributed over a region of about 85 kb of genomic DNA, overlapping the region of the *l'sc* gene; since some of these sequences are affected in the rearrangements, mutant phenotypes map throughout a very large chromosomal region. On the other hand, most, if not all, regulatory elements drive transcription of both T4 and T5 simultaneously. These two features explain most of the confusion that the genetic analysis has presented. In the field of genetic analysis of the AS-C, the question as to the specificity provided by the proteins is certainly a major challenge for further research. The AS-C has not yet given up all its secrets.

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