DEVELOPMENT IN GENETIC MOSAICS OF ARISTAPEDIA, A HOMOEOTIC MUTANT OF DROSOPHILA MELANOGASTER

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ABSTRACT

Development of the homoeotic mutation, aristapedia (ss), was investigated by means of genetic mosaics. The wild-type alleles of aristapedia and the bristle markers yellow, singed, and forked were removed from cells at different times in development by X-ray induced somatic crossing-over. The phenotype of the resulting clones was examined in order to ascertain whether it was leg or antenna. The y sn f; ss clones showed a leg phenotype if induced before the mid-third instar, but showed an antennal phenotype if induced after this time. Late non-expression of ss may be due either to an influence of surrounding ss+ tissues on the small ss clones, or to a persistence of the effect of ss+ for one or two cell generations after it is removed from a cell line.

HOMOEOTIC mutations appear to represent lesions in regulatory genes, since mutations in these genes cause one body part to develop into another. For example, aristapedia causes the distal part of the presumptive antenna to develop into the distal part of the leg (BALKASCHINA 1929) (see Figure 2), presumably by permitting leg genes to be used in what was previously antennal or uncommitted tissue (see POSTLETHWAIT and SCHNEIDERMAN 1973, for review). This investigation approaches the question of how a homoeotic gene effects this change in developmental pathways. LEWIS (1964) and ROBERTS (1964) have already shown that the wild-type alleles of bithorax and aristapedia are necessary in larval stages to prevent expression of the homoeotic phenotype. But GARCIA-BELLIDO and MERRIAM (1971) have demonstrated that for some genes the effects of an allele can persist for a few cell generations after that allele is removed from a cell by somatic crossing-over. So in this investigation we asked whether the effects of the wild-type allele of aristapedia could persist after it is eliminated from a cell line by somatic recombination. Since determination is heritable (HADORN 1967; BRYANT 1973), this technique should show when the final determination for leg vs. antenna occurs in the cephalic imaginal disc. If a change in genome fails to effect a change in phenotype, then determination has already occurred.

RATIONALE AND METHODS

X-ray induced somatic crossing-over was used to eliminate the dominant wild-type allele of

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Figure 1.—Somatic crossing over in the first chromosome of cell A, \(Dp(3;1)05, ss^+/\gamma sn f; ss^a/ss^a\) can produce daughter cells B and C. Cells of genotype C formed the clones examined in these experiments. Cells of type B could not be distinguished from those of type A.

aristapedia (\(ss^a, 3-58.5\)) from cells at various developmental stages. \(Dp(3;1)05, ss^+/\gamma; ss^a/ss^a\) males were mated to \(\gamma sn^3 f^{96a};ss^a\) females to provide the heterozygotes shown in Figure 1A. The marker genes are yellow (\(\gamma, 1-0.0\), yellow rather than black cuticle and bristles) singed (\(sn^a, 1-21.0\), twisted rather than straight bristles) and forked (\(f^{96a}, 1-56.7\), gnarled and forked rather than straight bristles). \(Dp(3;1)05, ss^+\) bears the wild-type allele of aristapedia translocated onto an otherwise wild-type first chromosome. Somatic recombination in cell A of Figure 1 can produce two daughters whose genotypes are shown in Figure 1B and C. Cell C is homozygous \(\gamma sn f\), and so cuticle made by cells of this genotype can be distinguished from that manufactured by cell A or B. In addition, cell C is homozygous \(ss^a\), having lost the dominant wild-type allele. Cell C or its progeny is then examined to see whether the phenotype corresponds to the genotype (leg) or not (antenna).

Parental flies were allowed to lay eggs for 12–24 hours on petri plates containing instant food (Carolina Biological Supplies). The eggs were permitted to develop specific times and were then irradiated with 1000 rads (.622 MeV, Gammatron M, Radiation International, Inc.). Precise ages were obtained by collecting irradiated animals as white prepupae every 4 hours. Time of irradiation is therefore given as hours before pupariation. After eclosion, the antennae of females were dissected, mounted in Gurr's Watermounting Medium between two cover glasses, and observed at 400X.

RESULTS

Figure 2 shows the phenotypes of a normal antenna, a wild type tarsus, and an aristapedia antenna. The arista is characterized by about ten long filaments in a plane, and about seven short filaments at right angles to this plane. The tarsus possesses bristles with sockets, and in addition, tarsus bristles have bracts formed by cells just proximal to the bristles. Apparently, the bristle induces the formation of a bract (POODBY, HALL and SUZUKI 1973; TOBLER 1969; TOBLER, ROTHENBUHLER and NÜTHIGER 1973). The claw organ terminates the leg. Bristles, bracts, and claws are never found on a normal arista. The arista of the aristapedia antenna is replaced by a tarsus, usually with a claw organ which is incomplete.
The results are based on a total of 1696 antennae from irradiated animals. These could be divided into three types. Type 1: Irradiation in the second or early third instar produced forty-three large clones of \( y sn f \) cells each of which displayed a clear leg phenotype (even at the edges) (Figure 3A). These clones were similar to those reported by Roberts (1964), and this fact indicates that \( ss^a \) tissue develops autonomously in large clones. Type 2: Irradiation in the early and mid-third instar provided forty-five small \( y sn f \) clones consisting usually of single leg bristles surrounded by antennal tissue (Figure 3B). These single bristles often had bracts, which were always on the proximal side of the bristle, as they are in the normal leg. In two of the observed clones the yellow singed forked leg bristle was accompanied by a black bract. It is not clear whether the black bract was a \( y sn f; ss^a \) leg cell which was caused to be black due to non-autonomy of yellow (Hannah 1953), or whether it was a heterozygous cell caused to be leg due to an inductive influence from the nearby genetically leg cell (see Garcia-Bellido 1972). Type 3: Irradiation in the mid- to late-third instar yielded twenty-five \( y sn f \) clones that showed an antennal phenotype—the aristal filaments (Figure 3C).

Figure 4 illustrates the frequency of marked clones with a leg or an antennal phenotype. When homozygosis for \( ss^a \) is induced within about twenty hours of pupariation, the clone does not express a mutant phenotype.
DISCUSSION

Early clones: These experiments show that an ss\textsuperscript{a} clone produced in the second or early third instar develops autonomously to produce leg tissue (Figure 3A and B). Therefore, the presence of the wild-type allele of aristapedia is necessary as late as the early third instar to prevent part of the cephalic imaginal disc from developing into leg tissue. The same is true for bithorax (Lewis 1964). This means that although developmental commitments may be initiated when the cleavage nuclei invade the blastoderm (see Bryant 1973; Gehring 1973; Postlethwait and Schneiderman 1973 for recent reviews), evidently genomic activity is necessary as late as the third instar to maintain or reaffirm the commitments.

Embryological and genetic evidence indicates that the mid-third instar is a period of intense developmental activity with regard to leg/antennal determi-
nation. First, the third instar is apparently critical in the development of leg/antennal homoeotic mutants since clonal analysis has demonstrated that cells in an Antennapedia antennal appendage retain the option to develop either leg or antenna until after the early third instar (Postlethwait and Schneiderman (1971b). Second, a genetically controlled process necessary for maintenance of normal determination is thermo-labile in the mid-third instar. Several conditional aristapedia alleles have been shown to have a temperature-sensitive period in the mid-third instar (Villeg 1942, 1943, 1966; Vogt 1946; Gloor and Kobel 1966; Grigliatti and Suzuki 1971), as have Nasobemia and Antennapedix, which may be Antennapedia alleles (Stepshin and Ginter 1972a,b). Finally, the leg/antennal developmental decision in the wild type is especially sensitive to environmental perturbations in the third instar. The wild-type

Figure 4.—Frequency of marked clones with a leg or antennal phenotype in relation to time of irradiation. The frequency of clones with a leg phenotype decreases in the mid-third instar, while the frequency of clones with an antennal phenotype increases in the mid-third instar. •, leg clones; □, antennal clones. At each age an average of 180 antennae were examined (range 28–403).
antennal disc can be caused to phenocopy the homoœcotic mutant by deleterious agents only during the third instar (Waddington 1942; Gersch 1946; Villée 1946; Bodenstein and Abdel-Malek 1949; Sang and McDonald 1954; Goldschmidt and Piternick 1957; Gehring 1964). Our results provide a basis for the above phenomena, since it is here shown that the $ss^+$ gene is required to prevent expression of a mutant phenotype until mid-third instar.

Late clones: Although early $ss^a$ clones developed autonomously, $ss^a$ clones induced in the late-third instar developed into antennal parts rather than leg tissue (Figure 3C). Three interpretations may explain this finding. First, the small mutant clones induced late in development may be non-autonomous due to influences emanating from adjacent wild type antennal cells. But, if the results were due to non-autonomy in small $ss^a$ clones caused by surrounding $ss^+$ tissue, then one might expect to find non-autonomy at the periphery of the large clones induced early. Yet neither Robert's (1964) experiments nor our studies produced a marked clone which was part leg and part antenna. In two cases we did see a black bract adjacent to a marked leg bristle, but this could have been non-autonomy of $yellow$ (Hannah 1953) rather than non-autonomy of aristapedia. Chimeras of $ss^a$ and $ss^+$ antennae produced mechanically by cell dissociation and reaggregation show autonomous differentiation even at the interface of $ss^a$ and $ss^+$ tissue (Garcia-Bellido 1968), except in rare cases which might be due to transdetermination (see Hadorn 1967; Gehring 1970). Hence, autonomous differentiation of leg and antennal cells seems to be the general rule.

A second interpretation is that the $ss^+$ allele effects a decision in the cell sometime prior to the late-third instar and once this event has occurred, even if the $ss^+$ gene is removed from the nucleus, cellular events proceed normally because the commitment has already been made. Only one or two proliferative cell divisions remain in the antenna after twenty hrs before pupariation (Postlethwait and Schneiderman 1971a); thus this second explanation calls for the effect of the wild-type gene to be retained only for one or two mitoses after the wild-type gene is removed from the clone. Evidence cited above in support of this viewpoint comes from temperature-sensitive mutants. Since the phenotype of temperature-sensitive aristapedia alleles cannot be altered in the late-third instar, apparently the state of the thermo-labile process is inconsequential after this time, and our data are consistent with the explanation that even the presence or absence of the gene itself is unimportant regarding determination after this time. The stability of the commitment after the mid-third instar is further illustrated by regeneration studies. Schübiger (1973) removed the antennal portion from late-third instar aristapedia cephalic discs and permitted regeneration to occur. He found that while wild type discs regenerated aristae, aristapedia discs regenerated tarsi.

A third explanation might be that following a change in the genome from $ss^+$ to $ss^a$ there is always a delay of a couple of cell divisions before the cytoplasm is sufficiently altered to change the cell's phenotype. Maybe, as Hadorn's (1967) dilution theory suggests, mitosis is necessary to dilute out $ss^+$ (antennal) determinants. This delay might occur not only after later irradiations but after early ones as well; but our procedure would not have detected a delay at earlier times.
To test this hypothesis one would need to metamorphose discs with $y$ sn $f$; ss$^a$ clones prematurely to find if even in young discs a couple of cell divisions are required to permit ss$^a$ expression.

**Polarity:** It has previously been shown that phenotypically leg cells surrounded by antennal cells can respond appropriately to patterning cues and polarity in Antennapedia antennal appendages (Postlethwait and Schneiderman 1971b). As Figure 3B shows, even in very small clones of genetically leg cells, bracts formed on the proximal side of bristles, as they do on the normal leg. This indicates that polarity relationships are not altered when a cell is caused to change from an antenna to a leg genotype. This could be either because the leg cell receives its polarity along with its cytoplasm from the mother cell, or that leg and antennal cells can both respond to the same positional information (Wolpert 1969). To distinguish between these two possibilities one must find and note the polarity relations of very small leg clones surrounded by antennal cells of dis-associated leg and antennal cells. Such experiments remain to be done.

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**LITERATURE CITED**


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