A CLONAL ANALYSIS OF DETERMINATION IN ANTENNAPEDIA A HOMOEOTIC MUTANT OF DROSOPHILA MELANOGASTER*

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Abstract.—The process by which cells select one of several specific programs of genetic information in development was examined in homoeotic mutant *Antennapedia of Rappaport* in *Drosophila melanogaster*. The results show that the alteration of determination from antenna to leg induced by this homoeotic mutation occurs in a population of cells related by proximity rather than by pedigree. Clones of cells marked by means of X-ray-induced somatic crossing-over prior to early third instar participated in the formation of both antenna and homoeotic leg, but after this time, the clone was restricted to either antenna or homoeotic leg, indicating that determination occurs in the third larval instar. The results are discussed in relation to other processes of determination.

Determination is the process by which cells select one of several specific programs of genetic information in a developing system. It has been regarded as a phenomenon essentially different from differentiation, which is the actual use of the selected program.[1] In some systems, the two processes can be studied independently, since they are widely separated in time. The imaginal discs of *Drosophila* provide such a system and have the added advantage that various genetic techniques can be used to investigate several determinative processes.

One of these determinative processes is the normal determination of the imaginal discs during embryonic and postembryonic life. A second process is transdetermination, which results in a change in developmental potential of imaginal disc cells during *in vivo* culture.[2] A third process occurs in homoeotic mutants, in which specific genes change the determination of imaginal discs during development. The present report examines the mechanisms underlying the determinative change in the homoeotic mutant *Antennapedia*. In this mutant, the antennal imaginal disc changes developmental programs, so that after metamorphosis mesothoracic leg structures appear in place of antennal structures. This mutant has a variable and capricious expressivity (Fig. 1), and the leg area may involve only a few cells in several separate regions of the disc or the entire antenna.[3] Specific antennal areas are transformed into specific leg regions. In the adult, proximal antennal segments are replaced by proximal leg parts, and distal antennal regions are replaced by distal leg structures.[3] Apparently specific regions of the antennal disc transform into corresponding regions of the leg disc, a phenomenon which will be discussed in a future communication.

The molecular mechanisms of determination are unknown, but a number of problems need to be solved as a prelude to biochemical approaches. The principal problem we wish to settle in this paper is whether the homoeotic alteration of determination occurs in a single cell, or more or less simultaneously in a popula-

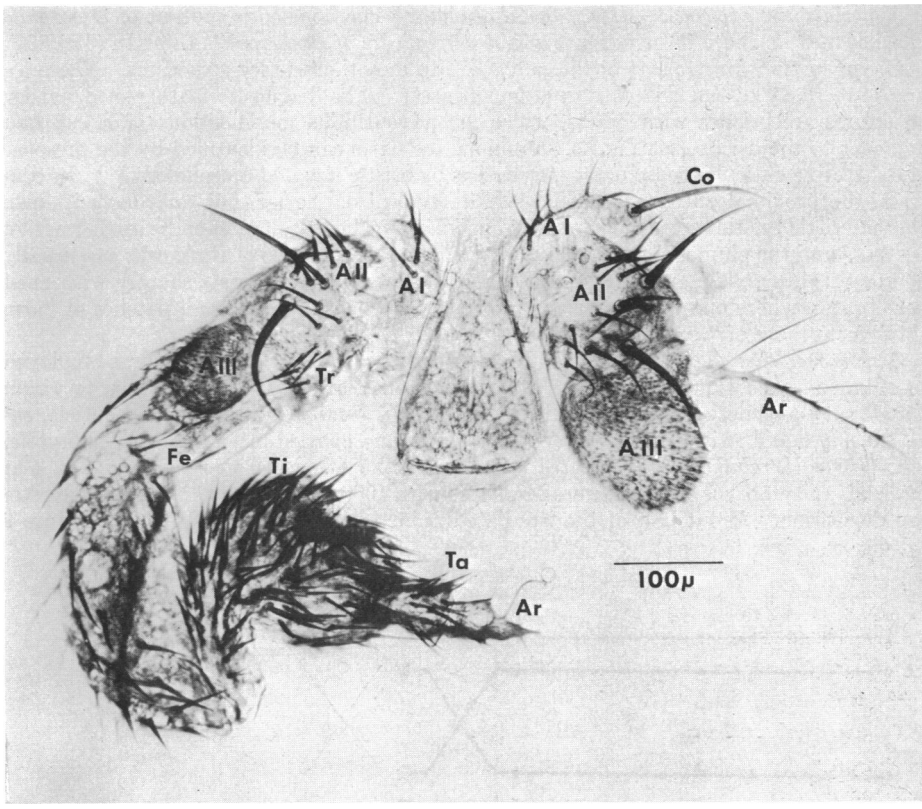

FIG. 1.—Antennae of a single $Antp^R/+$ individual. Notice the pronounced asymmetry. The left antenna of the fly is normal except for one leg structure, a coxal bristle (*Co*), which protrudes from the first antennal segment (*AI*). The right antenna shows a more extreme expression. Although the first antennal segment is normal, parts of the second antennal segment (*AII*) have been altered to produce trochanter structures (*Tr*). Also, most of the third antennal segment (*AIII*) and the base of the arista (*Ar*) have been modified into femur (*Fe*), tibia (*Ti*), and tarsus (*Ta*). Notice that proximal antennal segments produce proximal leg segments, and distal antennal segments produce distal leg segments, and that the homology extends even to parts of segments.

tion of cells. We also wish to learn the time of the determinative event. If the alteration of determination occurred in a unique stem cell, and this determined state is inherited by the progeny of this cell, then all the cells in the homoeotic leg would be the descendents of this single cell. This possibility is formally similar to the one suggested by Baker[4] and by Becker[5] in their analyses of variegation position effect and the pattern of defects in the mutant *Lobe*. An alternative possibility is that determination arises more or less simultaneously in a particular region in a population of cells that are related by proximity rather than by pedigree. Once determined, these unrelated cells would also pass to their progeny their specific determination. To distinguish between these two possibilities, we marked clones of cells by X-ray-induced somatic crossing-over, and proved that it is a population of cells and not a single cell which becomes determined to produce homoeotic leg.

Materials and Methods.—Experimental animals: The homoeotic mutant of *Drosophila melanogaster* we chose to investigate was *Antennapedia of Rappaport* ($Antp^R$ In (3R) 83F; 86C) wnich transforms parts of the antenna into mesothoracic leg structures. These leg areas are easily distinguished from antennal areas. The diagnostic feature of distal leg structures are bristles with bracts, which are adventitious modifications of a cell hair adjacent to the bristle (see Fig. 4). Proximal leg parts can be identified by the presence of distinctive cuticular sense organs, as well as by bristle size and morphology.[6, 7] In contrast, the proximal antenna bears no sensilla typical of the leg, but does have its own characteristic bristle morphology and arrangement. The distal antenna bears distinctive sensilla,[8] and the plumose arista. Thus, even an area of only two or three cells can usually be identified unambiguously as either leg or antenna. (This contrasts sharply with most vertebrates, where one would be hard pressed to distinguish very small patches of "arm skin" from those of "leg skin.")

Genetic methods: The technique of X-ray-induced somatic crossing-over was employed to obtain marked clones of cells. Males hemizygous for *y* sn^3 f^{36a} were mated to virgin females of the genetic constitution $Antp^R/Sb$ *Ubx* (*y* 1-0.0 *yellow* bristles and cuticle, sn^3 1-21.0 *singed* and twisted bristles, f^{36a} 1-56.7 *forked* and gnarled bristles, *Sb* 3-58.2 *Stubble*, *Ubx* 3-58.8 *Ultrabithorax*). Cultures were kept at 25°C. Eggs were collected at 4-hr intervals and embryos or larvae were irradiated with 1000 r of X-rays at specific times after egg deposition. About half of the irradiated animals pupated by 120 hr. As Figure 2

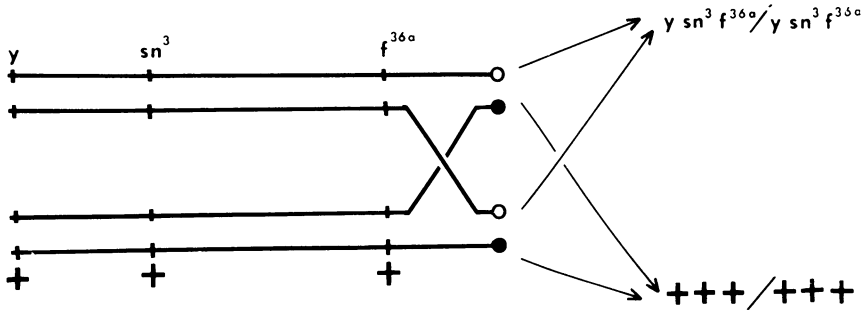

FIG. 2.—X-ray induced somatic crossing-over proximal to *forked* in the first chromosome of females heterozygous for genes affecting bristle color (*y*, *yellow*) and bristle morphology (sn^3, *singed-three;* and f^{36a}, *forked-36a*). Two types of progeny are produced: cells homozygous *y* sn^3 f^{36a}, which will show the mutant yellow gnarled bristle phenotype, and cells homozygous y^+ sn^+ f^+, which will show the wild-type, gray straight-bristle phenotype, and will be indistinguishable from the heterozygous *y* sn^3 f^{36a}/+ + + phenotype.

shows, somatic crossing-over proximal to *forked* on the first chromosome of heterozygous females, followed by cell division, yields a clone of cells homozygous for *y* sn^3 f^{36a} which will produce gnarled yellow bristles in the adult. Antennal appendages of *y* sn^3 f^{36a}/+++; $Antp^R$/+++ females were checked for clones of marked cells. The cuticular structures produced by these clones can tell us whether the determination to become homoeotic leg occurred (1) in a single cell which transmitted this new determination to its progeny, or (2) in a population of cells which occupy a certain region.

Predictions: If the homoeotic area is the progeny of a single cell, then genetic mosaics for bristle color and morphology induced *before* determination would yield some appendages in which the entire homoeotic region is marked with mutant, yellow and gnarled bristles (Fig. 3*A*). If bristle mosaics were induced *after* determination, then the clone of marked cells would be confined to either homoeotic or nonhomoeotic areas. However, if the homoeotic transformation occurs in a population of cells, then genetic mosaics for bristle color and morphology which are induced *prior* to determination could show both marked and unmarked tissue in both antenna and homoeotoic leg, and the mutant clone

would extend uninterrupted between the antenna and the homoeotic leg (Fig. 3*B*). *After* determination, the clone would be confined to either antennal or leg structures.

Results.—An analysis of 789 antennae from females irradiated between 3 and 137 hours after egg deposition indicated that hypothesis (2) was correct. Twenty appendages were found which had a clone in the antenna, but no marked leg structures, whereas no appendages were found having marked antennal cells which also formed a completely marked homoeotic leg. The lack of this class argues against hypothesis (1). There were 11 appendages, each bearing a single uninterrupted clone, which had marked and unmarked antenna, and marked and

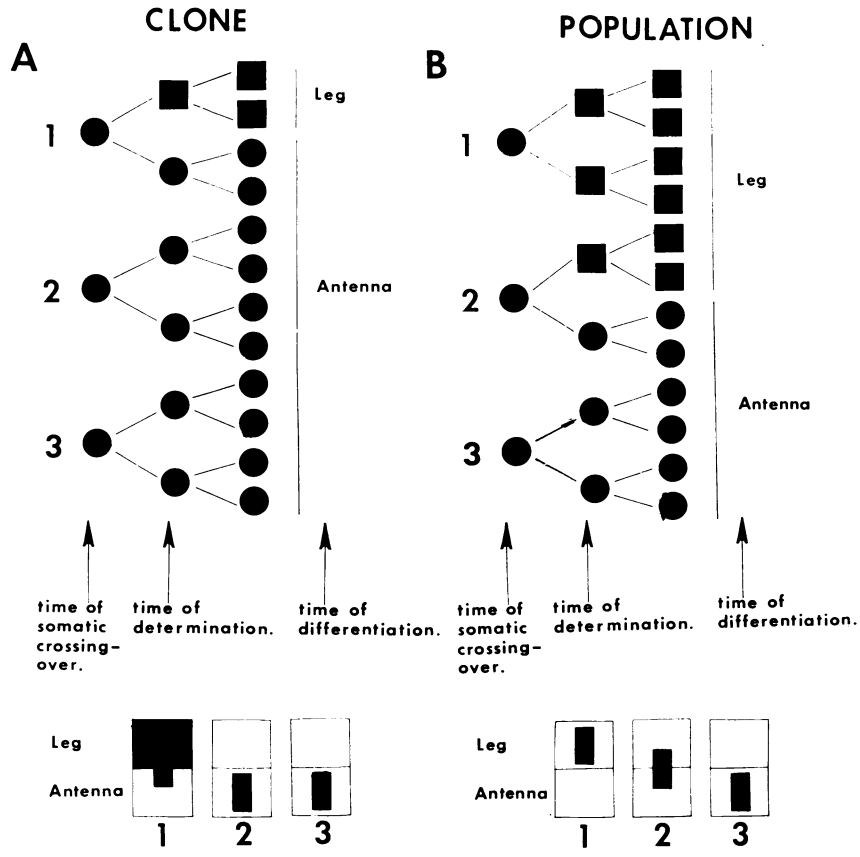

FIG. 3.—Experimental results predicted by the clonal hypothesis and by the population hypothesis of determination. The antenna is not a clone.[3] The upper part of the figure illustrates the possible fates of the progeny of three imaginal disc cells, 1, 2, and 3, which normally form part of the antenna. Antennal cells are represented as circles, whereas leg cells are represented by squares. If determination occurs in a *clone* of cells, (*A*) and cell no. 1 is marked by somatic crossing-over *before* determination, then part of the antenna and all of the leg will be marked. If cell no. 2 or 3 is marked, then part of the antenna, but none of the leg will be marked On the other hand, if determination occurs in *populations* of cells (*B*) and cell no. 1 is marked by somatic crossing-over *before* determination, then none of the antenna, and only part of the leg will be marked. If cell no. 2 is marked, then a continuous marked spot will extend from antenna into leg. If cell no. 3 is marked, then part of the antenna and none of the leg will be marked.

unmarked homoeotic leg, the result predicted by hypothesis (2). Figure 4 illustrates one of these 11 appendages which contains a clone participating in the production of both antenna and homoeotic leg. Notice that the clonally related cells in the $Antp^R$ antennal leg come to occupy longitudinal stripes, in the same fashion as in the thoracic leg.[9, 10]

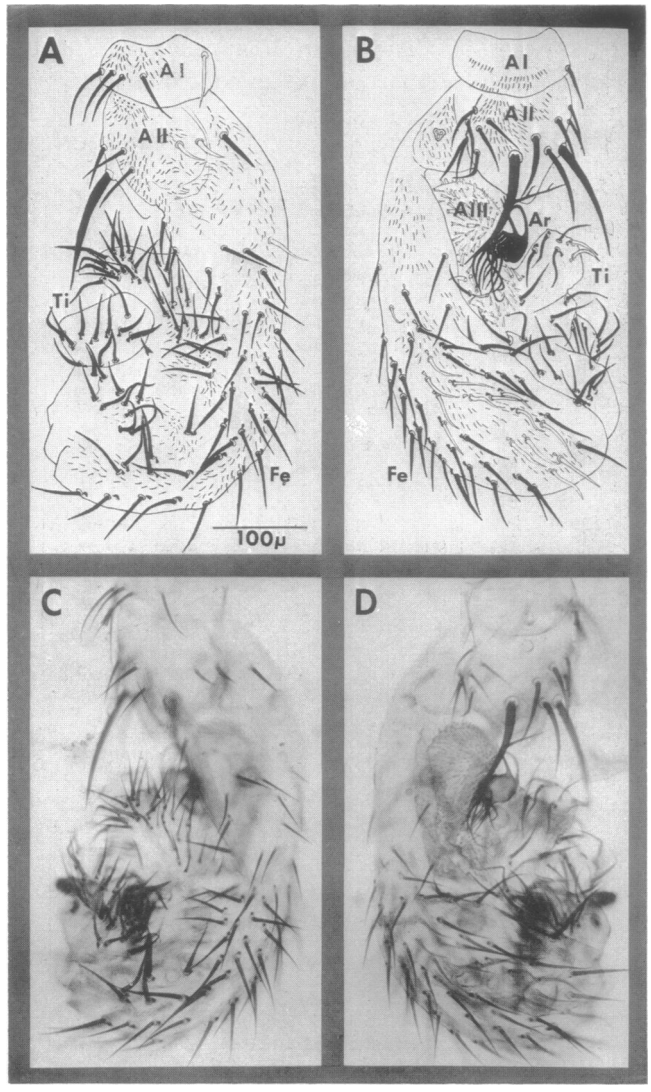

FIG. 4.—An *Antennapedia* antennal appendage mosaic for genes affecting bristle color and morphology induced at 56 hr after egg laying. (*A*) Camera lucida drawing, mesial aspect. (*B*) Camera lucida drawing, lateral aspect. (*C*) Photograph, mesial aspect. (*D*) Photograph, lateral aspect. Note one marked bristle on the first antennal segment, two marked bristles on the second antennal segment, 16 marked bristles on the femur, and two marked bristles on the tibia. The continuity of the clone between antennal regions and leg areas is apparent. This result is shown diagrammatically in Fig. 3*B* (2), and excludes the possibility shown in Fig. 3*A* (1). Therefore, determination influenced by $Antp^R$ occurs in populations of cells rather than in a clone of cells. Abbreviations are the same as Fig. 1.

Proof that the clones arose from single cells: Before hypothesis (2) can be accepted as proved, it is necessary to rule out the possibility that these 11 cases are due to independent somatic crossing-over events in more than one cell in an appendage. A Poisson analysis (see Table 1) revealed that of the 11 appendages with continuous marked patches only 3 can be accounted for statistically by crossing-over in two separate cells in the same disc. However, the *continuity of the clone* in all 11 appendages is strong evidence against a 2-cell origin of any of these 11 clones.

TABLE 1. *Distribution of clones.*

Age (hr)	Number of appendages examined	Average Number of Clones per Appendage		Expected number of appendages with more than one clone	Number of Appendages with Patches of Marked Bristles in *Both* Antenna and Homoeotic Leg	
		Experimental data	Poisson*		Interrupted patches†	Uninterrupted patches
12	135	0.007	0.005	0.01	0	0
24	267	0.034	0.033	0.03	0	1
41	122	0.098	0.103	0.61	0	4
56	126	0.13	0.14	1.1	0	5
72	60	0.53	0.51	5.4	3	1
95	28	0.61	0.62	3.6	1	0
118	22	3.2	3.0	17.8	11	0
137	28	2.2	2.2	18.2	3	0
					18	11

* The Poisson distribution of number of clones per appendage for each age group permits a prediction of the probability of having more than one somatic crossing-over event in an appendage. The fraction of appendages which had no clones was used to dictate each Poisson distribution because this class was unaffected by observational errors in scoring the number of clones per appendage. The experimental curve for each age group closely followed the corresponding Poisson distribution, and the experimental distribution mean was nearly equal to the calculated Poisson distribution mean.

† Additional appendages had more than one clone, but all were confined to either the leg or antenna.

The number of cells initially involved in the determination to produce homoeotic leg: By knowing the fraction of homoeotic leg produced by the clone of marked tissue in mosaics induced before determination, we can estimate the number of cells involved in the determinative event. (The assumption is made that all cells have similar division rates.) For example, in Figure 3 *B*, if the progeny of cell 2 is marked, then one third of the leg would be marked, indicating that three cells were determined to produce homoeotic leg. In our experimental mosaics which had a single clone participating in both antenna and homoeotic leg, an average of about one tenth of the leg bristles were marked. Therefore the determinative event which gave rise to homoeotic leg occurred in about ten cells.

The time at which the homoeotic gene alters determination: There were 18 appendages which had marked bristles in both antenna and homoeotic leg, but which lacked continuity between homoeotic and nonhomoeotic areas (Table 1). These all resulted from irradiation at 72 hours or later, and can all be accounted for statistically by independent somatic crossing-over events. The table shows that mosiacs induced later than 72 hours contain no clone which was continuous between antenna and homoeotic leg. Before 72 hours, the progeny of a single cell can participate in the formation of both a leg and an antenna, however, after 72 hours, no clones were detected which included both antenna and homoeotic leg.

From this, we conclude that the time of determination influenced by $Antp^R$ is after 72 hours, the beginning of the third instar. It is worth noting that this time of determination is much later than the normal time of determination of leg and antennal discs in wild-type embryos, which occurs prior to six hours after egg deposition.[11] In contrast, the cells in the antennal disc of $Antp^R$ animals retain the option to produce leg structures as late as the early third instar. Interestingly, this corresponds to the temperature-sensitive period of *aristapedia*, another homoeotic gene which transforms part of the antenna into part of the leg.[12, 13] Apparently, the time of action of *Antennapedia* corresponds to the presumed time of action of *aristapedia*. It is not clear whether the homoeotic change in determination transforms determined antennal cells to determined leg cells, or whether it changes cells from a general leg-antenna determination to specific leg determination or antenna determination, but we favor the former notion.

Discussion.—The present study confirms the hypothesis that the homoeotic gene *Antennapedia of Rappaport* alters determination in a population of cells related by proximity rather than by pedigree. In mosaics for bristle color and morphology which were induced prior to 72 hours, a marked clone of cells could produce both homoeotic leg and nonhomoeotic antennal areas. The clone was uninterrupted, and both homoeotic and nonhomoeotic regions contained marked and unmarked cells. The regional factors which actually cause the alteration in determination in homoeotic mutants are unknown, but there are several possibilities.

One possibility has been suggested by Kobel[14] based on his analysis of the location of homoeotic wing areas protruding from the eye of *loboid-ophthalmoptera* (ld^{oph} 3-102). He found that these areas were similar to the cell lineage sectors of the eye which were previously established.[4, 5] Kobel[14] suggested that this situation could occur if a clone of cells died, followed by rapid cell proliferation which increases the frequency of transdetermination.[15] Then, according to his scheme, a specific transdetermination could occur which involved cells, not necessarily clonally related, and eye tissue would be changed into wing structures. This explanation is unlikely for the general case, because regional cell death does not necessarily lead to proliferative overgrowth in other discs,[16] and there are no cases of transdetermination exclusively to a given structure.[2] Kobel's suggestion does not apply to $Antp^R$ because the pattern of expression of $Antp^R$ bears no consistent relationship to the known cell lineage of the wild-type antenna.[3] Therefore, it appears that homoeotic mutants change determination more or less simultaneously in a population of cells in a particular region, by a mechanism other than clonal cell death.

A second possibility is that a diffusible substance or a regional change in membrane conductivity[17] is responsible for the change of determination in a population of cells. If this were the case, mosaics of homozygous wild-type tissue in a background of heterozygous-dominant homoeotic tissue would yield wild type cells participating in the formation of homoeotic areas. Such nonautonomy has not been observed in mosaics of the *bithorax* alleles,[18] or in mosaics of *aristapedia*.[19] However, mixtures of wild-type leg discs or homoeotic antennal discs with wild-type antennal discs have yielded nonautonomous differentiation in border re-

gions,[20] indicating that homoeotic cells may have transformed their wild-type neighbors. The possibility that genetic mosaics of leg-antenna homoeotic mutants could yield nonautonomous differentiation is presently under investigation.

The homoeotic alteration of determination shares significant similarities with two other processes of determination in *Drosophila*, normal embryonic and postembryonic determination,[9] and transdetermination:[21] in all three processes, populations of nonclonally related cells are involved. Two processes of determination in man, the determination of red cells,[22] and the determination of certain types of malignant tumors[23] also are known to occur in populations of cells. Since the process of determination occurs in a regional group of cells, it seems likely that intercellular communication might play a role in the process of determination, a matter which is now being explored.

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