THE RELATIONSHIP BETWEEN THE FUNCTIONAL COMPLEXITY AND THE MOLECULAR ORGANIZATION OF THE *ANTENNAPEDIA* LOCUS OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

The *Antp* locus is involved in the development of the thorax of the larval and adult Drosophila. The absence of $Antp^+$ function during embryogenesis results in the larval mesothorax exhibiting characteristics of the prothorax and an ensuing lethality; the loss of $Antp^+$ function in the development of the adult thorax causes specific portions of the leg, wing and humeral imaginal discs to develop abnormally. Every *Antp* mutation, however, does not cause all of these developmental defects. Certain mutant alleles disrupt humeral and wing disc development without affecting leg development, and they are not deficient for the wild-type function required during embryogenesis. Other *Antp* mutations result in abnormal legs, but do not alter dorsal thoracic development. Mutations of each type can complement to produce a normal adult fly, which suggests that there are at least two discrete functional units within the locus. This hypothesis is supported by the fact that each of the developmental defects arises from the alteration of a different physical region within the *Antp* DNA. These observations indicate that the complete developmental role of the *Antp* locus is defined by the spatial and temporal regulation of the expression of several individual functional units.

EACH body segment of the *Drosophila melanogaster* larva and adult expresses a characteristic set of cuticular structures (such as antennae and wings) and the specific set that is expressed depends in part on the expression of genes within the Bithorax Complex (BX-C) and/or the Antennapedia Complex (ANT-C). The identities of those segments that compose the posterior portion of the fly, from the metathorax through the eighth abdominal segment, are determined by genes of the BX-C (LEWIS 1978; SANCHEZ-HERRERO *et al.* 1985), whereas the identities of the first two thoracic segments (prothorax and mesothorax), as well as the development of the gnathal segments of the head (mandibular, maxillary and labial), result from the expression of genes within the ANT-C (KAUFMAN 1983 and unpublished observations). The ANT-C and the BX-C appear to be individually responsible for the identities of the head and abdomen, respectively. However, the proper identities of the thoracic segments depend on the expression of specific loci within both gene complexes

(STRUHL 1982; KAUFMAN and ABBOTT 1984). Therefore, the analysis of the function of these particular genes and how they interact to promote normal thoracic development, be it directly or indirectly, should provide insight into the processes by which the developmental roles of the BX-C and ANT-C are defined.

The *Antennapedia* (*Antp*) locus is one member of the Ant-C that is involved in the normal development of the larval and adult thorax. Embryos which are deficient for $Antp^+$ function fail to make a normal mesothoracic segment, such that larvae developing from these embryos possess a second thoracic segment expressing characteristics of the prothorax (WAKIMOTO and KAUFMAN 1981). Likewise, the elimination of $Antp^+$ function, either during embryogenesis or the larval stage, results in the abnormal development of specific regions of the adult mesothorax, as well as the prothorax and metathorax (STRUHL 1981; KAUFMAN and ABBOTT 1984). The regions of the adult thorax which require $Antp^+$ function include portions of all three pairs of legs and specific regions of the dorsal mesothorax.

Clones of mesothoracic leg tissue which are deficient for $Antp^+$ function since early in embryogenesis are transformed to antennal tissue, suggesting that $Antp^+$ function promotes a mesothoracic identity by the suppression of head development (STRUHL 1981). The scope of this $Antp^+$ function is not, however, confined solely to the mesothorax. The transformation of prothoracic and metathoracic leg tissue to antenna in somatic clones which are deficient for the wild-type function of either the *Sex combs reduced* (*Scr*) or the *Ultrabithorax* (*Ubx*) loci, as well as $Antp^+$ function, has resulted in the hypothesis that $Antp^+$ function is actually required in all thoracic segments to suppress head development (STRUHL 1982). Furthermore, based on morphological criteria, DUNCAN (1982) and STRUHL (1983) have suggested that *Antp* is expressed in the first seven abdominal segments, but is apparently nonfunctional in the presence of BX-C gene expression.

The purpose of the aforementioned investigations was to define the primary developmental role of the *Antp* locus; consequently, the mutant alleles which were utilized in these studies were characterized as being completely deficient for its wild-type function. Animals which possess *Antp* null alleles terminate development at the end of embryogenesis, or shortly thereafter, and exhibit the mesothorax to prothoracic transformation described above. Not all *Antp* alleles, however, produce these early developmental defects (DENELL 1973; LEWIS *et al.* 1980b). Nevertheless, the effects of these particular *Antp* alleles could be accounted for if it were assumed that they reduce the level of, but do not eliminate, $Antp^+$ function; *i.e.*, they are hypomorphic alleles according to the critera of MULLER (1932).

The preliminary characterization of several new X-ray-induced *Antp* alleles showed that there were significant qualitative differences in the genetic behavior of various *Antp* alleles (ABBOTT and KAUFMAN 1983). As a result of these observations, a detailed developmental-genetic investigation of a number of *Antp* alleles was undertaken. The results of this study, which are presented in this report, suggest that the differential genetic behavior exhibited by various

Antp alleles is due to the mutation of independent functional units which reside within the locus. Based on the nonoverlapping developmental defects caused by different sets of these alleles, we suggest that the *Antp* locus is composed of at least two discrete functional units. Some of these alleles are associated with chromosomal rearrangements; therefore, it has been possible to correlate each of the functional units with a specific physical region within the *Antp* DNA.

MATERIALS AND METHODS

Culture conditions: All fly stocks were maintained at 25° on media consisting of cornmeal, molasses and agar. This media was supplemented with live baker's yeast.

Mutant chromosomes used: For clarity, the following nomenclature has been adopted in order to distinguish between the dominant and recessive *Antp* mutations: a recessive allele is designated with a superscript beginning with a lower case "*r*" and followed by a specific mutant designation (*e.g.*, $Antp^{rW10}$ or *rW10*), and a dominant allele possesses a superscript without the lowercase "*r*" (*e.g.*, $Antp^{CB}$ or *CB*). The *Antp* mutations utilized in this investigation are listed in Table 1. All mutations not referenced within this report may be found in LINDSLEY and GRELL (1968). The *Blunt-short bristles* (*Bsb*) mutation is described by BAKER (1980).

X-ray mutagenesis: To recover mutations in the *Antp* locus, a typical F_2 mutagenesis screen was employed. Males homozygous for the third chromosome mutations *red* (*3*-53.6) and *ebony* (*3*-70.7) were X-irradiated with a dose of approximately 4000 r. These males were then mated with virgin females heterozygous for the third chromosome balancers *TM3* and *CxD*. Between 40–50 females and 25 irradiated males were allowed to mate in bottles. After 2–4 days, the females were transferred to bottles containing fresh media and the males were discarded. From the F_1 progeny, either * *red e/TM3* (* *red e* designates the mutagenized chromosome) or * *red e/CxD* virgin females were collected and mass mated with *Df(3R)Scr* p^p e^s*/TM3* males. Several days later, single females were transferred to shell vials. The resulting F_2 progeny from each female was scored for the presence of the * *red e/Df(3R)Scr* individuals. If no * *red e/Df(3R)Scr* progeny were recovered, then a stock of that * *red e* chromosome was established using the * *red e/TM3* progeny.

Polytene chromosome cytology: The polytene chromosomes from all of the X-ray-induced mutant stocks were examined to determine if they possessed visible rearrangements. These chromosome preparations were made from the salivary glands of * *red e/red e* third-instar larvae. The mutant heterozygous larvae could be distinguished by the red pigmentation of their Malpighian tubules caused by the *red* mutation. These animals were raised at 18° in uncrowded conditions to maximize larval growth. The chromosome preparations were made according to the method of LEFEVRE (1976) and were examined with the phase contrast optics of a Zeiss Photomicroscope III.

Complementation studies: Complementation crosses were performed in order to establish allelic relationships between the X-ray-induced mutations. Typically, 10–15 virgin females were mated with 5–10 males in half-pint bottles. Unless specified, progeny were raised at 25°. Scoring of the P_1 progeny began on about the ninth day and continued for the next 6–7 days. A minimum of 100 progeny were scored for each cross.

Somatic recombination experiments: Somatic clones homozygous for $Antp^r$ mutations were generated by X-ray-induced mitotic recombination. Males carrying an $Antp^r$ allele were mass mated with virgin females heterozygous for *TM3* and a chromosome carrying the mutations *Kinked* (*Ki*), *pink-peach* (p^p), *Minute(3)*w^{124}, *Prickly* (*Pr*) and *Blunt-short bristles* (*Bsb*). Progeny from this cross were collected for either 12- or 24-hr intervals on 60 × 15 mm Petri dishes containing standard media. At various times after the midpoint of the collection period, the embryos or larvae were irradiated with 500

TABLE 1

The *Antp* mutations used in this investigation

Mutant chromosome	Cytology	Reference
Df(3R)Scr p^p e^s	Df(3R)84A1,2-84B1,2	LEWIS *et al.* (1980a)
Df(3R)Antp^{Ns+R17}	Df(3R)84B1-84D11,12	DUNCAN and KAUFMAN (1975)
In(3R)Antp^B p^p bx	In(3R)84B1,2;85E	LEWIS (1956)
In(3R)Antp^{73b} red e	In(3R)84B1,2;84C6	HAZELRIGG and KAUFMAN (1983)
In(3R)Antp^{Ns+RC4} e	In(3R)84B1;75A1,2	STRUHL (1981)
In(3R)Antp^{rS2} red e	In(3R)80;84B1,2	SCOTT *et al.* (1983)
In(3LR)Antp^W	In(3LR)75C;84B1,2	SCOTT *et al.* (1983)
In(3R)Antp^{CB}	In(3R)84B1,2;99F-100A	SCOTT *et al.* (1983)
In(3R)Antp^{RM}	In(3R)82E1;84B1,2	SCOTT *et al.* (1983)
T(2;3)Antp^{rK4}	T(2;3)36C,D;84B1,2 and In(3LR)62B;98F	LEWIS *et al.* (1980a)
T(2;3)Antp^{Ns+RC8}	T(2;3)41;84B1,2	STRUHL (1981)
Antp^{rR4} red e	Normal	LEWIS *et al.* (1980a)
Antp^{rW10} red e	Normal	WAKIMOTO and KAUFMAN (1981)
Antp^{rW24} red e	Normal	WAKIMOTO and KAUFMAN (1981)
Antp^{rS1} red e	Normal	SCOTT *et al.* (1983)
Antp^{rF9} p^p	Normal	P. FORNILI and T. KAUFMAN (unpublished results)
Antp^{rF22} p^p	Normal	P. FORNILI and T. KAUFMAN (unpublished results)
Antp^{rF36} p^p	Normal	P. FORNILI and T. KAUFMAN (unpublished results)
Antp^{rR40} p^p	Normal	P. FORNILI and T. KAUFMAN (unpublished results)
Antp^{rR10}, Ki p^p	Normal	LEWIS *et al.* (1980a)
Antp^{rK5}, Ki p^p	Normal	LEWIS *et al.* (1980a)
Antp^{rD7}, Ki roe p^p	Normal	LEWIS *et al.* (1980a)
Antp^{rR17}, Ki roe p^p	Normal	LEWIS *et al.* (1980a)
Antp^{50}	Normal	LINDSLEY and GRELL (1968)

r (50 r/min, 5 mA, 150 kV, 2-mm aluminum filter) delivered by a Torrex 150 X-ray machine (Torr Company, Los Angeles, California). After eclosion of the irradiated progeny, the *Antp^r/Ki p^p M(3)w^{124} Pr Bsb* animals were collected and stored in 95% ethanol. They were subsequently examined with a dissecting microscope to select those animals which possessed *Antp^r/Antp^r* somatic clones. The *Ki*, *Pr* and *Bsb* mutations greatly reduce the length of most macrochaetae, thereby facilitating the identification of the *Antp^r/Antp^r* clones which contain wild-type bristles. Animals with somatic clones were either examined with phase contrast optics or with a scanning electron microscope. Preparation of adult body parts for light microscopy was performed according to the method of SZABAD (1978). These body parts were mounted under coverslips with either Gurr Hydramount or AQ Mount (Bio/Medical Specialities, Santa Monica, California).

Lethal phase determinations: A number of crosses were performed to determine the lethal effects of various *Antp* mutations. In a typical experiment, ten *Antp^r/TM6B* males were mated with 15–20 *Antp^r/TM6B* virgin females. Eggs were collected over a 6- to 12-hr interval on 60 × 15 mm Petri dishes filled with agar/molasses media. Soon after the end of the collection period, 100–200 eggs were counted and transferred to a fresh collection plate. Sometime between 36- to 48-hr after the end of the collection period the number of hatched *vs.* unhatched eggs was determined. All unhatched eggs were dechorionated with bleach in order to count the number of unfertilized eggs.

TABLE 2

The X-ray-induced *Antennapedia* mutations

Antp mutation[a]	Cytology
rA58	Normal
rA60	Df(3R)84B2;D3
rA71	Normal
rA74	In(3R)84B2;87C
rA75	Df(3R)84B2-C6
rL1	T(2;3)84B2;25F
rL2	Normal

[a] All of these mutations were induced on a chromosome bearing *red* and *ebony*.

Hatched, first-instar larvae (whether dead or alive) were counted and transferred to a Petri dish containing standard media. At the onset of pupation, pupae were counted and transferred to another Petri dish containing a piece of moistened Whatman no. 1 filter paper and were allowed to eclose. A *TM6B* chromosome marked with *Tubby* (2-90.0) as described by CRAYMER (1984) was utilized in these experiments. The dominant *Tubby* mutation results in larvae and pupae which are shorter and fatter than wild-type pupae (CRAYMER 1980). Since the Tb^+ progeny from a mating between $Antp^r/TM6B$ individuals will be the $Antp^r/Antp^r$ animals, the *Tb* mutation permits the identification of this class at pupation. The number of animals reaching the pupal stage, pharate adult stage and eclosing was recorded. No attempt was made to score for lethality during specific larval instars.

RESULTS

Intra-allelic complementation between various *Antp* mutations: Seven X-ray-induced recessive lethal alleles of the *Antp* locus were recovered from a screen for lethal mutations in genes belonging to the ANT-C. Complementation tests between these seven lethal mutations and recessive lethal alleles of the genes within the ANT-C had shown that these X-ray-induced mutations only failed to complement recessive lethal mutations of the *Antp* locus (ABBOTT 1984). These "new" *Antp* alleles are listed in Table 2 along with the type of chromosomal rearrangement, if any, that was found in association with each of them.

In order to verify that these X-ray-induced mutations were actually allelic with each other, *inter se* complementation crosses were conducted. Included in these experiments were two X-ray-induced *Antp* mutations, *rS1* and *rS2*, which had been recovered from a previous screen (SCOTT *et al.* 1983). The results of these tests are presented in Table 3. All nine of the X-ray-induced lethal mutations failed to complement the *rW10* mutation, previously characterized as an *Antp* null allele by WAKIMOTO and KAUFMAN (1981). However, only the *rA74* and *rL2* mutations exhibited a complementation pattern identical to that of the *rW10* allele. In contrast to the *rA74* and *rL2* mutations, all of the other X-ray-induced mutations differed from the null allele in that they were found to complement both the *rA58* and the *rS1* mutations. In all but one instance, the P_1 mutant heterozygous progeny appeared at the expected frequency and

TABLE 3

The results of the *inter se* complementation crosses between the X-ray-induced *Antp* mutations

	rW10	*rA74*	*rL2*	*rA75*	*rA71*	*rS2*	*rL1*	*rA60*	*rA58*	*rS1*
rW10	—									
rA74	L	—								
rL2	L	L	—							
rA75	L	L	L	—						
rA71	L	L	L	L	—					
rS2	L	L	L	L	L	—				
rL1	L	L	L	L	L	L	—			
rA60	L	L	L	L	L	L	L	—		
rA58	L	L	L	V	V	V	V	V	—	
rS1	L	L	L	V	V	V	V	V	SL	—

At least 100 F_1 progeny were scored for every cross. L = mutant heterozygotes were not recovered; V = the expected frequency of mutant heterozygotes were recovered; SL = mutant heterozygotes were recovered at less than the expected frequency.

TABLE 4

The results of complementation crosses between the *rA58* and *rS1* Mutations and various *Antp* mutations

Antp mutations which failed to complement the *rA58* and *rS1* mutations	*rF22, rF36, rF40, rD7, rR17, rK4, rK5, rW24*
Antp mutations which complemented the *rA58* and *rS1* mutations	*rR4, rF9, B, 73b, W, 50, CB, RM*

with a wild-type phenotype. Only the *rA58/rS1* heterozygous individuals were found to exhibit a reduced level of viability.

As a result of the observed intra-allelic complementation involving the *rA58* and *rS1* alleles, additional complementation tests were conducted using these two alleles and a number of other recessive and dominant *Antp* alleles (Table 4). The dominant *Antp* alleles cause the homeotic transformation of the antennae to mesothoracic legs, and a majority share a common recessive lethality (DENELL *et al.* 1981). As shown in Table 4, both the *rA58* and *rS1* mutations were unable to complement a number of the recessive lethal alleles tested; however, further instances of intra-allelic complementation were observed. Significantly, all six of the dominant alleles tested were found to complement the recessive lethality of the *rA58* and *rS1* alleles. In most of these cases of intra-allelic complementation, the expected number of mutant heterozygous F_1 progeny were recovered, and they exhibited a wild-type phenotype; those progeny carrying a dominant allele also expressed the homoeotic transformation. Interestingly, many *rF9/rA58* and some *B/rA58* individuals exhibited a mutant prothoracic leg phenotype. This mutant phenotype will be described below.

The results from these complementation studies showed that all *Antp* mutations could be differentiated into two separate classes on the basis of the ability of each allele to complement the recessive lethality of the *rA58* and *rS1*

TABLE 5

The frequency and distribution of the X-ray-induced *Antp*^{r} somatic clones

Antp^r alleles	No. of *Antp*^{r}/*Ki* p^p *M(3)w*^{124} *Pr Bsb*, animals scored	Observed frequency of *Antp*^{r} clones	Distribution of *Antp*^{r} clones			
			Head	Dorsal thorax	Ventral thorax	Abdomen
rW10	3249	0.07	21	5	70	127
rA71	630	0.11	4	2	24	41
rR4[a]	4009	0.28	25	16	70	ND
rF9	2972	0.08	20	13	49	156

[a] The *rR4* clones were induced during the first larval instar; all other clones were induced during embryogenesis.
ND, not determined.

alleles. The members of that class of alleles which were unable to complement *rA58* or *rS1* are likely to be effectively deficient for all *Antp*$^+$ function since this group includes complete deletions of the locus (ABBOTT 1984). In contrast, the remaining alleles retained enough wild-type function such that, when combined with the defect expressed by the *rA58* and *rS1* alleles, a sufficient level of normal function was achieved to support proper larval and adult development. These observed cases of complementation between the *Antp* mutations could be attained in one of two ways, either as a result of the simple addition of reduced levels of the same product or through the contribution by each allele of an unaltered, discrete function to reconstitute the complete wild-type function of the locus.

***Antennapedia* is only required for the development of a discrete portion of the dorsal thorax:** One method commonly utilized to assess the role that a particular gene has in the development of the adult fly is the technique of X-ray-induced mitotic recombination (reviewed by POSTLETHWAIT 1978). This technique was utilized to generate somatic clones which were homozygous for the *Antp* allele *rW10*. These somatic clones were induced by X-irradiation of *rW10*/p^p *M(3)w*^{124} *Pr Bsb* embryos (6–18 hr after oviposition) or larvae (24–48 hr) and could be recognized in the adult because they possessed wild-type bristles as opposed to the mutant bristles in the surrounding tissue. The dominant mutations *Ki*, *Pr* and *Bsb* resulted in extremely shortened bristles. Clone size was increased by the presence of the *Minute* mutation, as described by MORATA and RIPOLL (1975).

The number and distribution of the *rW10* somatic clones which were recovered are shown in Table 5. It was observed that the loss of *Antp*$^+$ function during embryogenesis or the larval stage resulted in the abnormal development of specific regions of both the ventral and drosal thorax, but not of the head or abdomen. Specifically, the *Antp*$^-$ clones found in all three sets of legs were often associated with an aberrant morphology (see Figure 1D). This mutant phenotype consisted of a partial deletion of the tibia and the concomitant fusion of the femur and the basitarsus. When mesothoracic leg clones were induced early in embryogenesis (3–9 hr), the clonal tissue was found to be transformed to an antennal identity (ABBOTT 1984). This transformation was

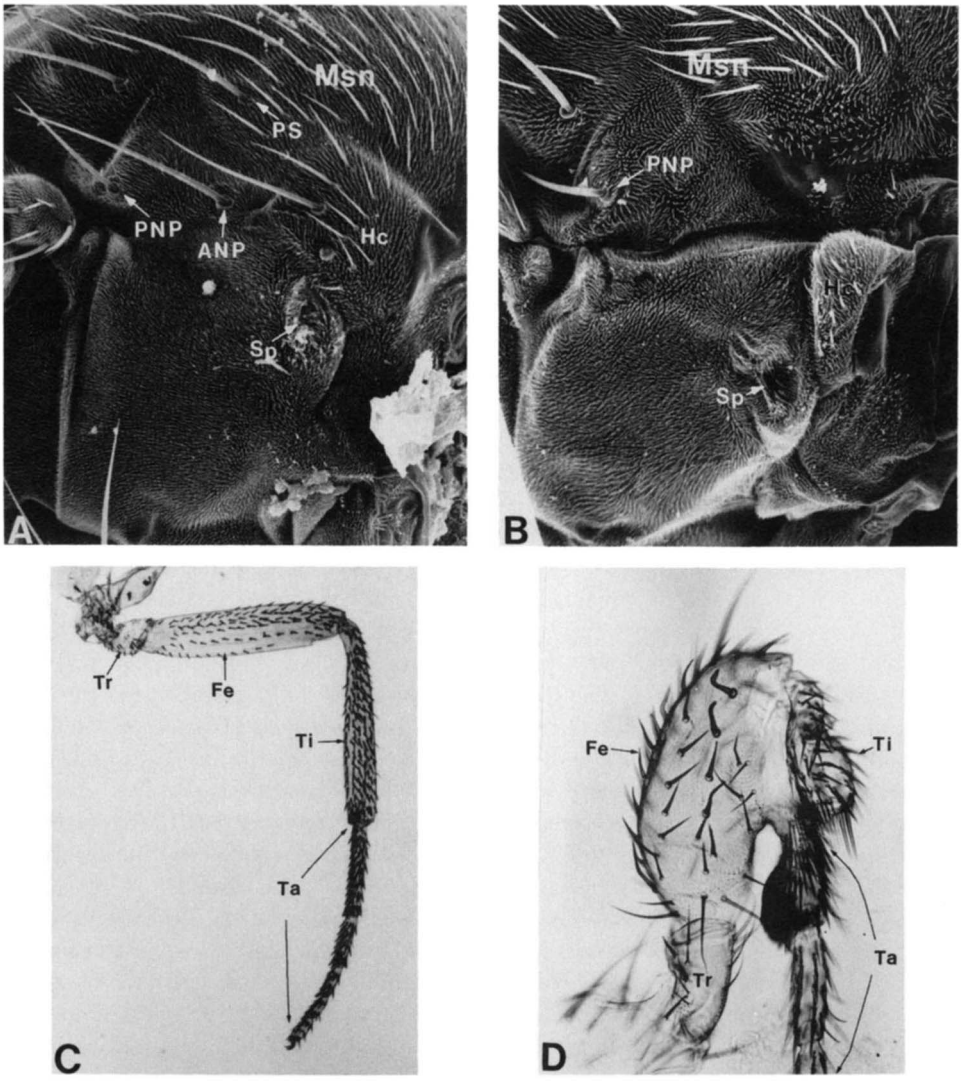

FIGURE 1.—Adult thoracic defects associated with *rW10/rW10* somatic clones. A, Cuticular region of the anterior mesonotum and humeral callus of a wild-type adult. B, Same region, but contained within a *rW10* clone induced during embryogenesis (6–18 hr). Several macrochaete are absent, and the pattern of microchaete is atypical. The morphology of the humeral callus is abnormal, but the normal complement of bristles is present. C, Morphology of a wild-type leg. D, Prothoracic leg that contains a *rW10* clone induced during embryogenesis (6–18 hr). The tibial morphology has been severely altered, and the femur is partially fused with the first tarsal segment. ANP = anterior notopleural bristle; Fe = femur; Hc = humeral callus; Msn = mosonotum; PNP = posterior notopleural bristle; Sp = anterior spiracle; Ta = tarsal segments; Ti = tibia; Tr = trochanter.

not observed in prothoracic or metathoracic leg clones, nor in mesothoracic leg clones induced after the middle of embryogenesis. All of these results are in agreement with those reported by STRUHL (1981).

TABLE 6

The number and distribution of mutant *Antp*^{r} leg clones

	Prothoracic legs		Mesothoracic legs		Metathoracic legs	
Antp mutation	Normal	Mutant	Normal	Mutant	Normal	Mutant
rW10	7	22	8	17	8	8
rA71	12	0	5	0	7	0
rR4	40	0	16	0	14	0
rF9	27	7	4	5	2	4

In contrast to these effects on the normal development of all legs, the examination of the dorsal thoracic *rW10/rW10* clones revealed that the loss of *Antp*$^+$ function prevented the development of only a restricted portion of the dorsal thorax. Specifically, the *rW10* clones which encompassed the region of the anterior mesonotum surrounding the humeral callus (Figure 1A) were always associated with alterations of normal cuticular morphology and the absence of both macrochaete and microchaete characteristic of this region. A typical *rW10/rW10* clone in the anterior mesonotum is illustrated in Figure 1B. In addition to the abnormalities of the anterior mesonotum, the normal morphology of the humeral callus was severely altered, although the usual complement of bristles was still present. Interestingly, *rW10* clones which did not extend into the anterior portion of the mesonotum were always completely normal, as were all wing clones recovered; therefore, the absence of all *Antp*$^+$ function after the middle of embryogenesis only affected the development of a restricted portion of the wing disc.

As a result of the complementation studies described above, it was concluded that many *Antp* alleles were not completely deficient for *Antp*$^+$ function. In order to ascertain what effects some of these alleles had on the development of the adult thorax, somatic clones homozygous for such alleles were generated and compared with those clones homozygous for the *rW10* allele. The number and distribution of the somatic clones homozygous for the different alleles which were tested are shown in Table 5. The distribution of the morphological defects associated with *rA71* and *rR4* thoracic clones differs from that of the *rW10* clones. As shown in Table 6, 47 of 70 *rW10* leg clones were found to be associated with the fusion of the femur, tibia and tarsal leg segments as described above, whereas no such mutant legs were observed in the 94 *rA71* and *rR4* leg clones which were recovered. Like the *rW10* clones, most of the *rA71* and *rR4* leg clones were found to fill either the entire anterior or posterior leg compartment; therefore, this difference in the effects of these alleles cannot be ascribed merely to a difference in clone size. Similarly, although the *rR4* clones were induced later in development than were the *rW10* clones in this particular experiment, this also did not account for the lack of *rR4* mutant leg clones. When *rW10* leg clones are induced as late as the third larval instar, they also exhibit this abnormal morphology (KAUFMAN and ABBOTT 1984).

While the *rA71* and *rR4* clones were not found to be associated with defective leg development, they did cause the same aberrant mesonotal morphology

as was found in association with *rW10* clones which encompassed the anterior mesonotum. There was no discernible difference between the mutant phenotype associated with the *rA71* or *rR4* clones and that associated with the *rW10* clones.

Somatic clones homozygous for the *rF9* allele were also examined. In contrast to the results obtained for the *rA71* and *rR4* alleles, the *rF9* leg clones were frequently associated with the mutant leg phenotype (see Table 6); however, the *rF9* mesonotal clones expressed only a relatively mild effect on the development of the anterior mesonotal region. For example, all *rW10*, *rA71* and *rR4* clones in this region lacked the presutural and notopleural bristles, but only 25% of the *rF9* mesonotal clones were found to share this defect; and none of these clones affected the humeral callus as did clones homozygous for the other alleles. Hence, the *rF9* clones are similar to the *rW10* clones in their effect on leg development, but they are unlike the latter in that they do not severely disrupt the morphology of the anterior mesonotum. In addition, even though the *rF9* mutation complements the *rA58* mutation as it does the *rA71* and *rR4* mutations (Tables 3 and 4), the *rF9* leg clones are unlike the leg clones of the latter two alleles in that they are often associated with a mutant phenotype.

Based on the complementation results, it was concluded that the *rA71*, *rR4* and *rF9* alleles were unlike the null allele *rW10*. The results from the mitotic recombination experiments confirmed this conclusion, because each of these alleles caused only a subset of the defects produced by the null allele. The fact that the *rA71* and *rR4* clones did not affect leg development suggests that it is possible to inactivate a wild-type function of *Antp* necessary for normal mesonotal development without the concomitant loss of a function required for leg development; that is, the *Antp* locus must possess at least two discrete functions in adult development. While it is possible to inactivate the function required for normal mesonotal development without affecting the function required for leg development, the reciprocal effect was not observed. The *rF9* clones did have a discernible effect on mesonotal development, although not as pronounced as that of the null allele.

The alteration of a different region within the *Antp* locus is responsible for each of the phenotypic defects: In addition to the *rA71* and *rR4* alleles, other *Antp* alleles belonging to the group with members that behave differently than the null allele are also deficient for the mesonotal function. This observation is based on experiments in which the *rF9* allele was heterozygous with $Df(3R)Antp^{rA60}$, $T(2;3)Antp^{rL1}$ or $In(3R)Antp^{CB}$. Occasionally, viable adults heterozygous for *rF9* and one of these mutations were recovered; these individuals always had normal legs, but expressed abnormalities of the anterior mesonotum and derivatives of the humeral disc. This phenotypic defect is described below. Consequently, this mutant phenotype suggested that the *rA60*, *rL1* and *CB* alleles are deficient for a dorsal thoracic function. However, they still retain the normal leg-specific function because the heterozygous mutant individuals possessed normal legs, even though the *rF9* allele is effectively deficient for

the leg-specific function, as demonstrated by the mitotic recombination experiments.

Even though a comparable number of F_1 progeny were scored *rF9/Df(3R)Antp^{rA75}* adults were not recovered (data not shown). Based on this result, it was concluded that the *rA75* deletion did not complement *rF9*, because it had deleted an *Antp*$^+$ function that was retained, at least partially, on the *rA60* deficiency chromosome. This followed from the fact that both deletions extend into the region of the *Antp* locus from its telomeric side, and from the assumption that it was improbable that both deficiencies shared a common proximal breakpoint; therefore, they would be partially nonoverlapping within or near the *Antp* locus.

The differential complementation behavior of the *rA60* and *rA75* deficiencies with regard to the *rF9* allele was also observed for the inversions *Antp^{CB}* and *Antp^{W}*. As noted above, *CB/rF9* adults were recovered, whereas *W/rF9* adults were not (data not shown). The inversion breakpoint of the *W* allele is proximal to the *CB* breakpoint within the locus itself (SCOTT *et al.* 1983); consequently, the *W* inversion effectively splits the locus into two halves (designated here as "proximal" and "distal," relative to the centromere and telomere; see Figure 4), inverting the proximal half into the left arm of chromosome *III*. The *CB* inversion, by contrast, breaks the locus near its distal boundary; therefore, much of the locus remains intact. Hence, the differential complementation behavior of the *CB* and *W* alleles, and perhaps the *rA60* and *rA75* deletions as well, could be attributed to the destruction of the *cis* relationship between the region delimited by the *CB* and *W* breakpoints and the proximal portion of the locus. Furthermore, since the *CB/rF9* adults possessed normal legs, it was hypothesized that some portion of the distal half of the locus was not required for normal leg development.

These observations suggested that the separable *Antp*$^+$ functions described above could actually be localized to discrete physical regions within the locus. This hypothesis was further supported by observations regarding the complementation behavior of the *rA58* allele. This allele complemented the recessive lethality of those alleles associated with rearrangement breakpoints mapping between the *W* and the *CB* alleles. However, the *rA58* mutation was originally recovered because *rA58/Df(3R)Scr* adults were not viable. The *Df(3R)Scr* mutation deletes all of the proximal half of the locus (SCOTT *et al.* 1983). It followed from these observations that breaking the *cis* relationship between segments of the locus within the distal half did not inactivate the *Antp*$^+$ function(s) affected by the *rA58* mutation, whereas the deletion of the proximal half of the locus had this effect.

Although *rA58/Df(3R)Scr* adults were not recovered, a large number of these individuals survived to the pharate adult stage (data not shown). Examination of these pharate adults, after their removal from the pupal case, revealed that they possessed an abnormal pair of prothoracic legs, but had normal mesothoracic and metathoracic legs (Figure 2). As shown in this figure, the abnormal prothoracic leg morphology displays the same defect observed in the *Antp*$^-$ leg clones described above. The failure of the *rA58/Antp*$^-$ mutant

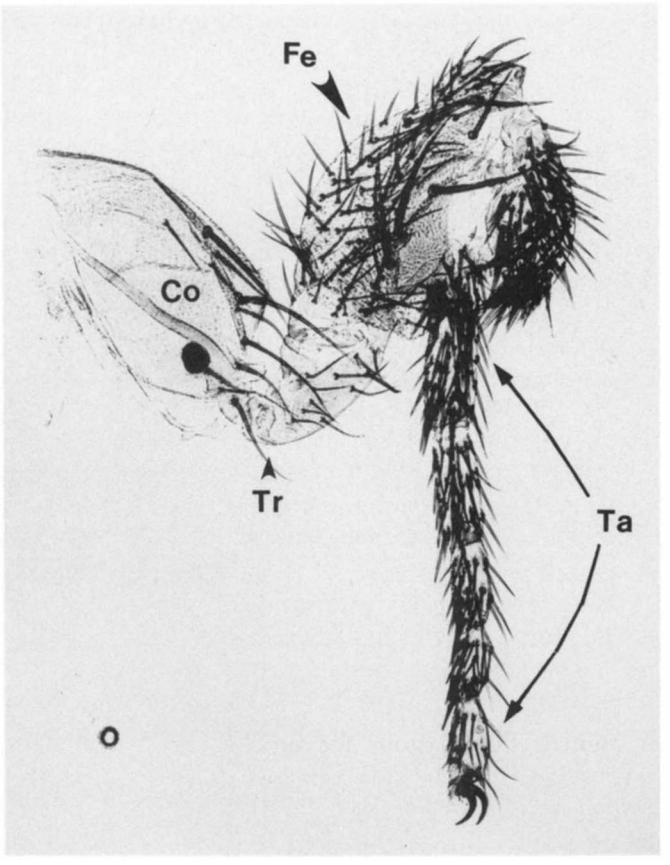

FIGURE 2.—Typical mutant prothoracic leg of a *rA58/Df(3R)Scr* pharate adult. Note the similarity in the mutant phenotype shown here and the mutant leg phenotype associated with the *rW10* somatic clones (Figure 1D). Co = coxa; Fe = femur; Ta = tarsal segments; Tr = trochanter.

individuals to eclose from the pupal case is most likely due to a mechanical difficulty derived from the dramatic crippling of the prothoracic legs. Indeed, individuals of this genotype dissected from the pupal case are capable of movement and will survive for several days after removal. Therefore, this failure to eclose should not be confused with the lethality to be discussed below. This same mutant leg phenotype was also observed in the complementation crosses involving the *rA58* mutation and the *rF9* and *B* mutations. Aside from its puzzling restriction to the prothoracic legs, this mutant phenotype (also found when *rA58* was paired with any *Antp* null allele) supported the hypothesis that the $Antp^+$ function required in the development of the leg was located in the proximal half of the locus. It should be noted, however, that the *cis* relationship between the proximal and distal regions is necessary for the dorsal thoracic function; therefore, both halves of the locus contain information required for this function.

A portion of the *Antp* locus is not required for larval viability: In the process of maintaining the *W/TM6B* stock it was observed that *W/W* animals

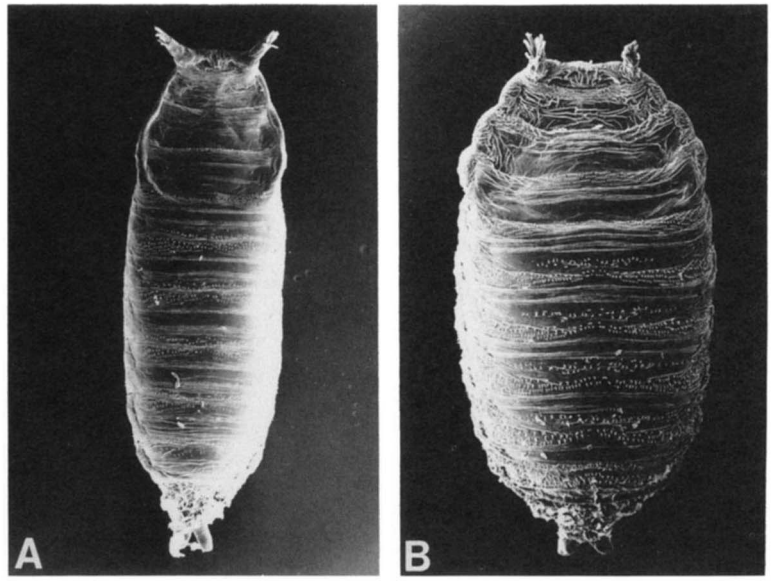

FIGURE 3.—The effect of the *Tubby* mutation on pupal morphology. A, A wild-type pupa. B, A +/*Tb* pupae.

frequently survived to the pupal stage. WAKIMOTO and KAUFMAN (1981) had observed that animals homozygous for an *Antp* null allele did not survive beyond the larval stage. The fact that animals homozygous for the *W* inversion, which splits the locus into two halves, survived beyond the larval stage suggested that the *cis* relationship between these two halves was not required for the proper expression of the $Antp^{+}$ function required during embryogenesis; *i.e.*, this rearrangement had not created a null allele. Consequently, this function could be localized to a specific physical region within the locus (either in the proximal or distal half) like the leg and wing imaginal disc-specific functions.

To test this hypothesis, the lethal phase was determined for the F_1 progeny heterozygous for the *rW10* allele and one of a series of *Antp* alleles for which associated breakpoints had been mapped within the locus. If a particular *Antp* allele was totally deficient for the embryonic function, then the mutant heterozygous progeny should fail to survive beyond the larval stage, as had been observed for the *rW10* allele. Conversely, those alleles not deficient for this function should allow survival beyond the larval stage, as had been observed for the *W* homozygotes.

In order to unambigously identify any *Antp* mutant heterozygous progeny surviving to the onset of pupation in these experiments, the third chromosome balancer *TM6B* was utilized. This balancer carries the dominant mutation *Tubby* (*Tb*), which makes *Tb*/+ pupae shorter and fatter than +/+ pupae (Figure 3); this phenotype is completely penetrant and expressive. In a cross between $Antp^{r}$/*TM6B* individuals, only the $Antp^{r}$ heterozygous pupae will be Tb^{+}. Con-

TABLE 7

The maternal effect of the *TM6B* chromosome on the viability of the F_1 progeny

	Genotype of parents[a]		Eggs		Larvae			Pupae enclosing (%)	
No.	Female	Male	No. collected[b]	Hatching (%)	No. collected	Pupating (%)	*Tb*$^+$/*Tb* pupae	*Tb*	*Tb*$^+$
1	+/+	+/+	931	94.7	478	92.9			
2	+/*TM6B*	+/*TM6B*	1278	76.4	470	81.1	0.29	36.6	22.1
3	+/+	+/*TM6B*	962	98.3	230	90.0	0.97	18.1	46.9
4	+/*TM6B*	+/+	915	88.2	378	85.5	0.99	27.4	44.8
5	+/+	*rW10/TM6B*	1130	98.0	557	96.8	0.94	33.0	41.1
6	*rW10/TM6B*	+/+	1432	75.5	533	83.0	0.94	17.2	38.9

[a] An Oregon-R third chromosome = "+."
[b] These values represent the number of fertilized eggs that were collected.

sequently, the ratio of *Tb* to *Tb*$^+$ pupae is a measure of the survival of the mutant heterozygotes to the pupal stage.

A series of control crosses were conducted to establish the level of survival to be expected of the F_1 progeny from the experimental crosses (Table 7). A comparison of the results obtained from these crosses showed that the level of survival of the F_1 progeny was significantly altered by the presence of the *Tm6B* balancer. A decreased hatching frequency was observed among the embryos obtained from +/*TM6B* or *rW10 red e*/*TM6B* mothers relative to the +/+ mothers (compare crosses 3 and 4, or 5 and 6). Based on this observed maternal effect, the level of lethality found in the fertilized eggs of +/*TM6B* parents (24%) could not be due entirely to the lethality of the *TM6B*/*TM6B* embryos. If the decreased level of survival of the embryos from +/*TM6B* females is due to the presence of *TM6B*, then the lethality of *TM6B*/*TM6B* embryos should be about 14%, which would be approximately one-half the value expected (25%) if all *TM6B*/*TM6B* embryos failed to hatch.

The presence of the *TM6B* chromosome in the female parent was also found to be correlated with a decreased level of larval survival (compare crosses 1, 3 and 5 with 2, 4 and 6). However, the expected ratios of *Tb*$^+$/*Tb* pupae were observed in all crosses, suggesting that the +/*TM6B* maternal genotype had a uniform effect on all progeny classes. This indicates that the ratio of *Tb*$^+$ to *Tb* pupae is a reliable indicator of larval viability.

The results from the experimental crosses are shown in Table 8. In all but two of the crosses *rW10 red e*/*TM6B* females were used; therefore, based on the results described above, about 65% of the fertilized eggs recovered from each cross were expected to hatch if the *Antp* mutation tested did not result in embryonic lethality. This value takes into account the fact that about 25% of the total number of fertilized embryos will succumb to the maternal effect and that 14% of the remaining embryos will die as a result of being homozygous for *TM6B*. The mean value for the hatching frequency was 67%, not including those crosses in which *rW10 red e*/*TM6B* females were not used

TABLE 8

Embryonic, larval and pupal viability of the F_1 progeny resulting from crosses between *Antp*$^-$/*TM6B* and *Antp*$^-$/*TM6B* individuals

No.	Allele tested[a]	Eggs: No. collected[b]	Eggs: hatching (%)	Larvae: No. collected	Larvae: pupating (%)	*Tb*$^+$/*Tb* pupae	Pupae enclosing (%): *Tb*	Pupae enclosing (%): *Tb*$^+$	*Tb*$^+$ pharate Adults (Y/N)[c]	Latest stage attained by *Tb*$^+$ progeny
1	*rL2*	409	68.5	171	77.8	0	48.5	0	N	Larval
2	*rA74*	1005	65.0	467	68.1	0	54.7	0	N	Larval
3	*RC8*	673	45.9	250	66.4	0	36.8	0	N	Larval
4	*B*	840	68.9	482	61.6	0	39.0	0	N	Larval
5	*rS2*	951	69.1	586	64.9	0.085	22.0	0	N	Pupal
6	*rA75*	865	66.1	484	63.2	0.108	29.5	0	N	Pupal
7	*W*	668	65.4	390	73.5	0.241	25.9	0	N	Pupal
8	*rS1*	590	69.5	277	77.9	0.245	26.7	0	Y	Pupal
9	*rA58*	1574	68.6	876	70.5	0.279	30.7	0	Y	Pupal
10	*rA60*	988	62.6	483	75.4	0.269	21.0	0	Y	Pupal
11	*rF9*	825	83.0	469	76.8	0.286	45.0	0	N	Pupal
12	*rL1*	928	26.1	191	71.2	0.198	37.4	0.5	Y	Adult
13	*CB*	919	89.0	609	89.0	0.273	19.5	0.8	Y	Adult

[a] Except for the crosses involving the *rF9* and *CB* alleles, *rW10 red e/TM6B* females were used in every case.

[b] These values represent the number of fertilized eggs that were collected.

[c] Y = yes; N = no.

(crosses 11 and 13) and the two which exhibited significantly reduced levels of embryonic lethality (crosses 3 and 12); all of the remaining crosses yielded hatching frequencies within two standard deviations from this mean. Based on the close agreement between the expected and observed levels of embryonic viability, it was concluded that (with the exceptions noted above), for the alleles which were examined, most of the *Antp*^{r}/*rW10* progeny did not exhibit a significant level of embryonic lethality.

The two crosses in which significant levels of embryonic lethality were observed involved the *rL1* and *RC8* alleles, both of which are associated with chromosomal translocations. Consequently, the low levels of embryonic viability can be ascribed to aneuploid embryos resulting from adjacent I and II disjunction; that is, eggs fertilized by aneuploid sperm. This conclusion is supported by the fact that the levels of embryonic lethality were similar among a number of crosses involving each of these translations (ABBOTT 1984).

Considerations of embryonic lethality aside, in most of the experimental crosses, a significant number of the *rW10/Antp*^{r} progeny survived beyond the larval stage, as evidenced by the fact that the ratio of *Tb*$^+$ to *Tb* pupae approached that of the control value; therefore, none of these alleles was deficient for the embryonic function. However, there were four instances where no *Tb*$^+$ pupae were recovered (crosses 1–4), indicating that individuals heterozygous for these alleles and *rW10* did not survive beyond the larval stage. Since complete prepupal lethality is a characteristic of the *Antp* null allele

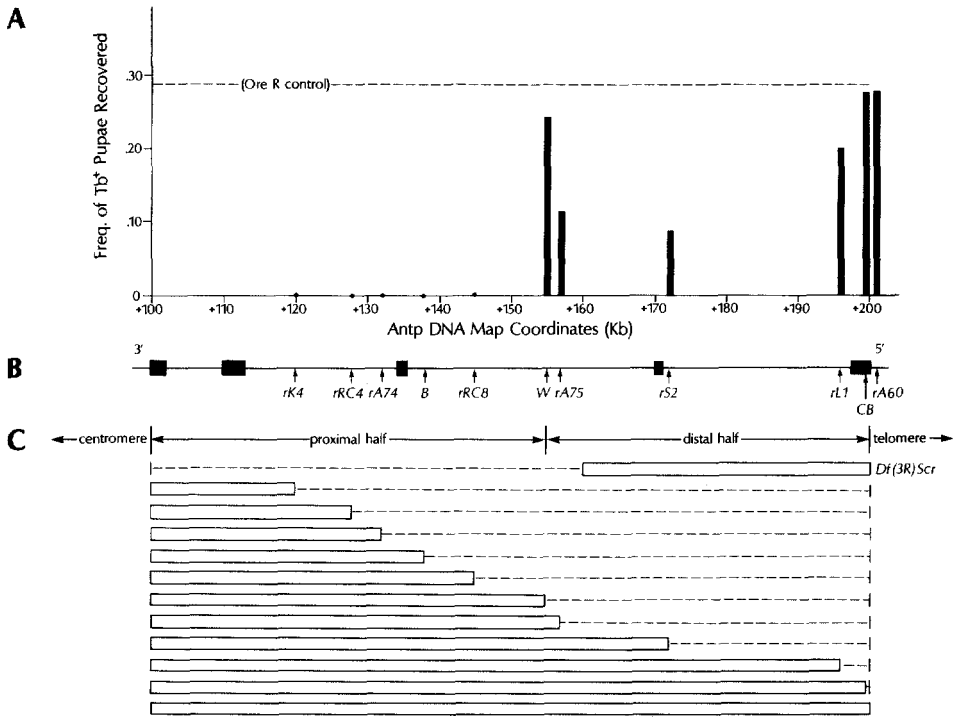

FIGURE 4.—The effect of various *Antp* mutations on larval viability. A, A histogram illustrating the results presented in Table 8. The height of each bar represents the frequency of survival of Tb^+ larvae to the pupal stage. The Tb^+ individuals are heterozygous for *rW10* and each of the *Antp* alleles that was examined. The position of each bar along the abscissa reflects the approximate location of the chromosomal rearrangement breakpoint within the *Antp* DNA. The dashed line is the level of larval viability obtained from the cross of Ore-R/*TM6B* individuals (see Table 7). Note that the data for the levels of survival of *rW10/rK4* and *rW10/rRC4* individuals is not shown in Table 8 but may be found in ABBOTT (1984). B, The relationship between the *Antp* mutations which were examined and the positions of the exons belonging to the *Antp* transcription unit (GARBER, KUROIWA and GEHRING 1983; SCOTT *et al.* 1983; S. HORIKAMI and T. KAUFMAN, unpublished results). Note that each bar of the histogram corresponds to one of the mutations shown. C, A schematic diagram illustrating the extent to which the chromosomal rearrangements which were examined alter the *cis* relationship between the proximal and distal halves of the locus. Each horizontal bar corresponds with one of the mutations shown; the length of each open bar indicates the amount of *Antp* DNA within each rearranged chromosome that remains contiguous with the 3′ end of the locus. The dotted line represents the *Antp* DNA that has been deleted or that remains contiguous with the 5′ end of the locus. The transition from the open bar to the dashed line marks the approximate location of the rearrangement breakpoint. The deletion of the proximal half on the *Df(3R)Scr* chromosome is also shown.

rW10, the *rL2*, *rA74*, *RC8* and *B* alleles would also appear to be null based on this criterion.

The larval viability values from each of the experimental crosses have been plotted against the physical location of the breakpoints associated with the allele examined (Figure 4). From this figure, it is clear that alleles which have breakpoints within the proximal portion of the locus are unable to provide the $Antp^+$ embryonic function required for larval viability, whereas those alleles with

breakpoints that are not located in this region are not deficient for this function. These results show that the *Antp*$^+$ embryonic function required for larval viability resides within the proximal half of the locus. In addition, the results from these experiments also support the hypothesis that the distal region of the locus is required for the development of only the dorsal thorax.

All of the *Tb*$^+$ pupae that were recovered exhibited defects in the development of the anterior spiracles by failing to evert these spiracles at the onset of pupation. Consequently, these mutant pupae had a characteristic blunt-ended appearance when compared to wild-type pupae (Figure 5A and C). the *rL1/rW10* and *CB/rW10* adults which were recovered from these mutant pupae exhibited an abnormal anterior mesonotum, like the *rF9/Df(3R)rA60* animals (Figure 5D). This mutant phenotype is characterized by a disruption of the normal morphology of the tissue surrounding the humeral callus and the anterior spiracle, and it differs from the phenotypic defects associated with the *rW10*, *rA71* and *rR4* somatic clones in that (1) the presutural and notopleural bristles are present, and (2) the large macrochaete normally found on the posterior portion of the humeral callus are always absent. In addition, *Df(3R)rA60/rW10* pharate adults also express this mutant phenotype, but none of these animals eclosed. Although most pupae possessing the spiracular defect did not continue to develop, the fact that viable adults could be recovered from such pupae suggests that this defect is not, by itself, responsible for the observed pupal lethality.

DISCUSSION

***Antennapedia* has several discrete developmental functions:** The objective of the investigations described in this report was to determine the effects of various *Antp* mutations on normal Drosophila development. The results of these studies show that not every *Antp* mutation causes the same set of recessive, developmental defects which are observed to occur as a result of a deficiency for the *Antp* locus; in other words, the complete elimination of all *Antp*$^+$ function. Specifically, it was found that the *Antp* mutant alleles which were examined could differ from one another with respect to two recessive characteristics: (1) the specific region of the adult thorax that they affect and (2) their ability to support development beyond the larval stage.

The complementation studies involving the X-ray-induced *Antp* mutations showed that some of these mutations exhibit complementation behavior like the null allele *rW10*; however, the genetic behavior of a number of them is dissimilar to the behavior of a null allele. The *rW10* allele fails to complement the *rA58* mutation; the heterozygous individuals do not eclose from the pupal case, although they often form pharate adults which possess mutant prothoracic legs. In contrast, many *Antp* mutations complement *rA58*, resulting in viable adults with normal prothoracic legs. This intra-allelic complementation behavior indicates that some *Antp* mutations, unlike a null allele, retain the wild-type function for which the *rA58* allele is deficient.

Examination of somatic clones which were homozygous for different *Antp* recessive alleles revealed that the mutations *rR4* and *rA71*, which are unlike

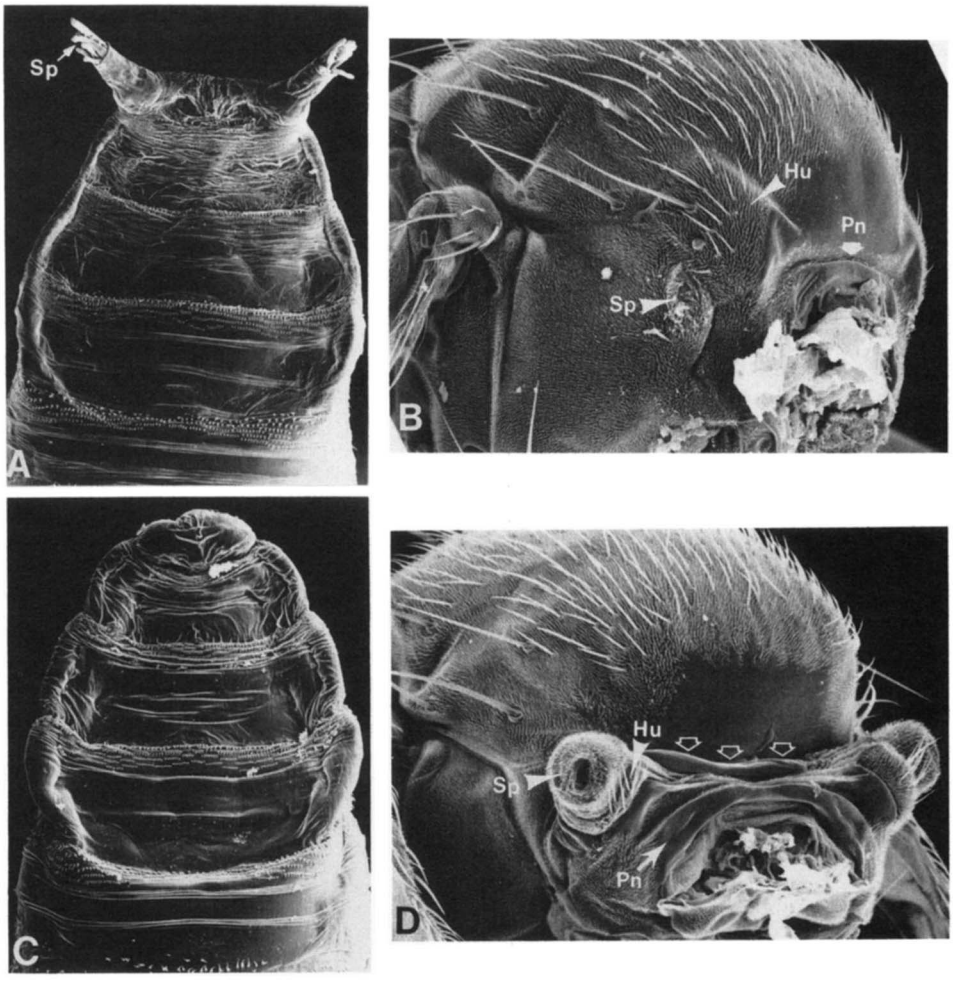

FIGURE 5.—Mutant pupal and adult phenotype caused by the *Antp* mutations which alter the distal half of the locus. A, Dorsal view of the anterior half of a wild-type pupae. B, Anterior-lateral view of the mesonotum and humeral regions of a wild-type adult. C, Dorsal view of the anterior of a *rF9/Df(3R)Antp*^{rA60} pupa. Note that the anterior spiracles (Sp) have failed to evert. D, The anterior-lateral mesonotum and the humeral region of a *rF9/Df(3R)Antp*^{rA60} adult. The arrows indicate the position of a band of cuticle that resembles the pronotum (Pn). The presence of this tissue occurs concomitantly with the absence of the posterior portion of the humeral callus (Hu).

rW10 in that they both complement *rA58*, are also unlike this null allele in their effect on the development of the adult thorax. The *rW10* allele disrupts the development of the derivatives of the leg, wing and humeral discs, whereas the *rR4* and *rA71* mutations do not affect leg disc development, although they disrupt wing and humeral disc development to the same extent as the *rW10* mutation. Consequently, it appears that neither the *rR4* nor the *rA71* allele is lacking the *Antp*$^{+}$ function required for normal leg development, but they are

completely deficient for an *Antp*$^+$ function involved in the development of the dorsal thorax.

The differential effect on thoracic development caused by the *rW10*, *rR4* and *rA71* mutations was also observed in the case of the *rF9* somatic clones. However, the *rF9* mutation expressed a different, and almost reciprocal, effect on thoracic development when compared with those defects resulting from the *rR4* or *rA71* mutations. The *rF9* leg clones were often abnormal, but the mesonotal clones were not, at least to a degree similar to the defects caused by the other mutations.

The mild effect of the *rF9* mutation in the development of the dorsal thorax may be due to (1) the inability to dissociate, by mutation, the leg-specific function from the mesonotal function or (2) unique properties of the *rF9* allele itself. For example, this allele is different from those mutations which complement *rA58*, because the *rF9/rA58* adults often possess abnormal prothoracic legs. Since this particular mutant phenotype is characteristic of pharate adults which are heterozygous for *rA58* and a null allele, it appears that the *rF9* allele differs from the null condition by permitting the survival of these mutant individuals. Hence, there may be a correlation between the mild effect on dorsal thoracic development and the survival of the *rF9/rA58* individuals; that is, the *rF9* allele represents an intermediate form of *Antp* allele possessing characteristics of both a null allele and an *rR4*-like allele. Nevertheless, the *rF9* clones, like the *rR4* and *rA71* clones, show that not every *Antp* mutation affects both the dorsal and ventral thorax to the same extent.

The lethal phase determinations indicate that mutation of the *Antp* locus can result in lethality at two discrete periods in development. Some mutations are unable to support development beyond the larval stage, which is a characteristic of *Antp* null alleles; therefore, they must eliminate the *Antp*$^+$ function required during embryogenesis. A number of *Antp* alleles which were tested, however, do permit the survival of animals to the pupal stage at levels equivalent to those of wild type. Clearly, these alleles are not deficient for the *Antp* embryonic function required for larval viability, but they do lack an *Antp*$^+$ function necessary for pupal viability.

The results of the investigations described above, when taken together, allow the conclusion that the *Antp* locus possesses several discrete developmental functions. These individual functions are required for (1) the identity of the larval mesothorax, as well as survival through the larval stage; (2) the eversion of the anterior spiracles at the end of the larval stage and survival through the pupal stage; (3) the proper development of all leg discs; and (4) the proper development of a portion of the wing and humeral discs. Since a subset of these functions can be eliminated without the concomitant loss of one or more of the others, it seems reasonable to conclude that the expression of these functions is, to varying degrees, autonomous of the expression of the others during the normal course of development.

Separate physical regions of the locus encode different developmental functions: Many of the *Antp* alleles examined in the lethal phase studies are associated with detectable alterations of the *Antp* DNA (see Figure 4). Based

on this information, it has been shown that the deletion of the distal region of the locus [*e.g.*, *Df(3R)rA60*] or the destruction of the *cis* relationship between the proximal and distal halves [*e.g.*, *In(3LR)Antp*^{W}] does not result in larval lethality, which would occur due to the absence of the *Antp* embryonic function. Therefore, the information necessary for this function must be completely encoded within that portion of the locus that lies proximal to the physical location of the *W* inversion breakpoint.

We refer to the portion of the locus containing all of the information necessary for the embryonic function as the embryonic "functional unit." The fact that some of the discrete *Antp*$^{+}$ functions depend on the *cis* relationship between the proximal and distal halves of the locus (see below) suggests that a functional unit may correspond to a transcription unit. If this relationship does exist, then the autonomous expression of *Antp*$^{+}$ functions might be attributable to the expression of different transcription units, throughout development.

The DNA composing the portion of the locus distal to the *W* breakpoint contains the 5′ end of a putative transcription unit that is made up of discrete regions distributed across the entire locus (GARBER, KUROIWA and GEHRING 1983; SCOTT *et al.* 1983). Since the expression of the embryonic functional unit is not affected by the juxtaposition of non-*Antp* DNA with the proximal region, at or distal to the *W* breakpoint, this putative transcription unit cannot correspond to the embryonic functional unit. Consequently, a second transcription unit may reside within the DNA located proximally to the location of the *W* inversion breakpoint. Further characterization of the details of the expression of the *Antp* locus at the molecular level, however, will be required to determine the validity of this hypothesis, as well as ascertaining if the *Antp* functional units do in fact correspond to different transcription units.

Regardless of how the *Antp*$^{+}$ embryonic function is expressed, it is clear that a similarly restricted portion of the locus is also both necessary and sufficient for the leg-specific function in adults. When the *cis* relationship between both halves of the locus is destroyed, the function required for normal leg development is not necessarily eliminated, whereas those alleles associated with physical alterations in the proximal third of the locus do lack this function (ABBOTT 1984). It has also been observed that the *rA58* mutation, which itself disrupts leg development, does not complement *Df(3R)Scr*, a deletion of the entire proximal half, but does complement *Df(3R)rA75*, which deletes a significant portion of the distal half. This shows that the *Antp*$^{+}$ function necessary for leg development maps to the proximal region. Finally, the *rS1* mutation is associated with the insertion of a transposable element at map position +134 (SCOTT *et al.* 1983); a position that lies within the proximal region. If this inserted piece of DNA is responsible for the phenotypic defects caused by the *rS1* mutation, then the fact that these defects are identical to those caused by the *rA58* mutation further implicates the proximal region of the locus as the domain containing the leg-specific functional unit.

All but two of the *Antp* alleles which result in the homoeotic transformation of the antennae to mesothoracic legs are associated with chromosomal rearrangement breakpoints which lie within the distal half of the locus (GARBER,

KUROIWA and GEHRING 1983; SCOTT *et al.* 1983). The apparent restriction of these breakpoints to only this part of the locus suggests that the homoeotic phenotype is due to the improper expression of the leg-specific functional unit in the eye-antennal disc, since the normal effect of this $Antp^{+}$ function is to promote leg development. With the exception of the *B* allele, all of these dominant *Antp* mutations fully complement the inability of the *rA58* allele to support prothoracic leg development; therefore, they are not deficient for the leg-specific function. Interestingly, *B/rA58* adults occasionally exhibit abnormal prothoracic legs, indicating that the *B* inversion does affect the expression of this function. This effect may be related to the fact that the *B* inversion breakpoint of all of those rearrangements causing the dominant homoeotic transformation is located in the closest proximity to the region of the locus containing the information necessary for the leg-specific function (see Figure 4). Consequently, this inversion may cause a reduction in the level of expression of the leg-specific function due to the proximity of the inversion breakpoint to the DNA encoding this function; that is, the occasional mutant phenotype exhibited by *B/rA58* individuals results from a position-effect.

The improper expression of the leg-specific function within the eye-antennal disc cannot simply arise as a result of the elimination of a *cis* relationship between that part of the locus that encodes this function and a distally located region containing information necessary for its proper expression. Several of the X-ray-induced mutations possess rearrangement breakpoints with physical positions within the *Antp* DNA that are interspersed with those breakpoints associated with the homoeotic transformation (Figure 4), yet these X-ray-induced alleles do not result in a detectable antenna to leg transformation.

The homoeotic transformation may depend on the exact nature of the non-*Antp* DNA sequence(s) that is juxtaposed with the remaining portion of the locus as a result of a chromosomal rearrangement (SCOTT *et al.* 1983). That the constitution of the non-*Antp* DNA can influence the expression of information encoded within the proximal region is illustrated by a consideration of the *rS2* inversion. This inversion is broken within the distal region and results in the juxtaposition of the proximal region of *Antp* with centromeric heterochromatin. The presence of this heterochromatin may be responsible for the fact that fewer *rS2/rW10* individuals survive through the larval stage than would be expected solely on the basis of the physical location of the inversion breakpoint (Figure 4); therefore, the expression of the embryonic functional unit is reduced due to this juxtaposition of heterochromatin.

Due to the preponderance of chromosomal rearrangements which cause the dominant homoeotic transformation as opposed to those which do not, it might be argued that the precise composition of the non-*Antp* DNA, juxtaposed by the rearrangement, is not the crucial factor in the generation of the homoeotic phenotype. However, it should be noted that this apparent superiority in the number of those rearrangements causing the homoeotic transformation is in fact a sampling artifact; that is, mutations which cause this phenotype are easily detected and readily saved (and have been for several decades), whereas rearrangement breakpoints within the locus, but not associated with the dominant

phenotype, are only recovered by screening for their recessive lethality. Consequently, the juxtaposition of "permissive" non-*Antp* DNA sequences which result in the improper expression of the leg-specific function in the eye-antennal disc is most probably a very rare event. This suggests that the juxtaposition of only highly specific DNA sequences can alter the segmental specificity of this function.

While the proximal half of the locus is sufficient for both the embryonic and leg-specific functional unit(s), it is not the case that the distal half alone can supply the functional unit(s) required for pupation and normal dorsal thoracic development. Animals homozygous for the *W* inversion die during pupation and possess noneverted anterior spiracles, indicating that the *cis* relationship between the proximal and distal halves is required to suppress these defects. Therefore, the larger known *Antp* transcription unit could correspond to the functional unit required for dorsal thoracic development as well as pupal viability.

The proper expression of each of the $Antp^+$ functions depends on information contained within the proximal half of the locus. Since the null alleles are, by definition, deficient for all of these functions, it must be that either all, or part, of this information is shared by all of the *Antp* functional units. Therefore, it seems reasonable to expect the alterations of the *Antp* DNA associated with these null alleles to be clustered within the proximal half of the locus. The exact locations of these clusters would then indicate specific sites within the proximal region which encode the information necessary for every $Antp^+$ function. It is instructive to note that the *Antp* homoeobox DNA sequence lies within this proximal region (LAUGHON and SCOTT 1984).

The results of the developmental-genetic investigations presented in this report suggest that the *Antp* locus possesses several discrete functions which are required for proper thoracic development. Based on information regarding the molecular organization of this locus, it has been possible to show that the expression of each of these individual functions is dependent on information contained within unique portions of the locus. Consequently, the complete developmental role of the *Antp* locus would appear to be defined by the proper spatial and temporal expression of each of these unique regions during the course of development.

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