GENETIC ANALYSIS OF THE ANTENNAPEDIA GENE COMPLEX (ANT-C) AND ADJACENT CHROMOSOMAL REGIONS OF *DROSOPHILA MELANOGASTER*. I. POLYTENE CHROMOSOME SEGMENTS 84B-D

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ABSTRACT

A deficiency spanning section 84B-D of the proximal right arm of chromosome 3 of *Drosophila melanogaster* has been utilized as a screen for mutations that aid in the genetic dissection of this developmentally interesting region.—— Ninety-two mutations have been recovered, of which 12 have been localized by deficiency and complementation mapping to the 84B1,2 doublet, the site of the Antennapedia complex (ANT-C). This has permitted a more precise description of the genetic organization of this complex locus.——A collection of 31 mutations that reside in 84C1-D2 displays an intricate circular complementation pattern, characterized by two clusters of mutations that exhibit semi-lethality, negative complementation and temperature sensitivity. Mutations affecting seven additional functional groups within the 84B-D region were also recovered.

HOMOEOTIC mutations are characterized by a striking perturbation of normal development in which one structure is formed in place of another. The suggestion that at least some of these mutations may represent loci involved in key determinative decisions during development (WADDINGTON 1940) has provided the rationale for many studies of homoeosis. (For reviews see OUWENEEL 1976; GARCIA-BELLIDO 1977.) Although homoeotic mutations are distributed throughout the genome, at least two important clusters of related loci are known. The pioneering studies of E. B. LEWIS (1963, 1978) have focused on the bithorax gene complex (BX-C), which is located in polytene chromosome region 89E and includes a series of tightly clustered loci that control determinative decisions affecting the mesothorax and all segments posterior to it.

A second cluster of homoeotic mutations is located in the polytene chromosome

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interval 84A-84B. Cytogenetic and genetic studies of this more proximal group indicate that it contains genes affecting the development of the head and thorax, leading to the hypothesis that it represents the anterior analog of the BX-C. If region 84A-84B does contain such a homoeotic gene complex, we anticipate that its constituent loci would show physical clustering, functional relationships and interactions analogous to those of the BX-C (KAUFMAN, LEWIS and WAKIMOTO 1980).

At the initiation of the present investigation, this putative homoeotic complex (ANT-C) was identified only by mutations that yield viable and homoeotically transformed adults and revertants of $Antp^{Ns}$ (DENELL 1973; DUNCAN and KAUFMAN 1975; KAUFMAN 1978; KAUFMAN, LEWIS and WAKIMOTO 1980). Based on the results involving the BX-C (E. B. LEWIS 1978), we would predict that many mutations yielding homoeotically transformed larvae would be lethal. Thus, in this report and the accompanying paper (LEWIS *et al.* 1980), an attempt has been made to saturate the 84A-B region with new mutations, the recovery of which does not depend on adult viability.

A number of new mutations has been recovered based on their inability to complement $Df(3R)Antp^{Ns+R17}$, which deletes polytene chromosome region 84B-D. An analysis including deficiency and complementation mapping not only elucidates the genetic organization of this portion of the ANT-C, but identifies additional interesting loci in sections 84C-D, where the visible mutations roughened eye (*roe*), rotund (*rn*), (DUNCAN and KAUFMAN 1975) and a valine tRNA (DUNN *et al.* 1979) have previously been localized.

MATERIALS AND METHODS

All flies were raised on standard cornmeal, molasses, brewer's yeast and agar medium. Crosses were performed at 25° ± 1°, except for those crosses used to obtain larval salivary glands for preparation of polytene chromosomes, which were carried out at 18° ± 1°. A description of the various marker mutations and balancer chromosomes can be found in LINDSLEY and GRELL (1968). Mutations of particular interest are listed in Table 1.

TABLE 1

Mutations and chromosomes utilized in this study

Homoeotic mutations	Symbol	Cytology	Transformation
Antennapedia^{7sb}	$Antp^{7sb}$	aberrant in 84C-D	antenna → leg II
Antennapedia^{B}	$In(3R)Antp^{B}$	*In(3R)84B1,2;85E*	antenna → leg II
Antennapedia^{50}	$Antp^{50}$	extra band near 84B1,2	antenna → leg II
Antennapedia^{60}	$Antp^{60}$	extra band in 84B1,2	antenna → leg II
Antennapedia^{R}	$In(3R)Antp^{R}$	*In(3R)83F; 86C*	antenna → leg II
Antennapedia$^{Extra\ Sex\ Combs}$	$Antp^{Scx}$	normal	leg II,III → leg I
Antennapedia^{Nasobemia}	$Antp^{Ns}$	normal	antenna → leg II

TABLE 1—Continued

Homoeotic mutations	Symbol	Cytology	Transformation
Humeral	*In(3R)Hu*	*In(3R)84B2–3; 84F2–3; 86B4–C1*	sternopleural bristles on propleurae propleura → mesopleura
Multiple Sex Combs	*In(3R)Msc*	*In(3R)84B1,2;84F*	leg I → leg II leg II,III → leg I
proboscipedia	*pb*	normal	labial palpus → leg I or antenna
Thickened Arista	*T(2;3)Ta²*	*T(2;3)51E1,2; 84B2–3*	arista → tarsus

Deficiency chromosomes	Discoverer	Cytology	Sites exposed
Df(3R)Antp^{Ns}+^{R17}	DUNCAN & KAUFMAN	*Df(3R)84B1; 84D11–12*	*Scr, Msc, Antp, Hu, tRNA^{Val}, rn, roe*
Df(3R)Scr	SINCLAIR	*Df(3R)84A1,2; 84B1,2*	*pb, Scr, Msc, Antp, Hu*
Df(3R)Antp+^{R1P}	GREEN	*Df(3R)84C3–4; 84D* + a point mutation at *Antp*	*Antp, roe, rn*
Df(3R)Antp+^{R2}	GREEN	*Df(3R)84B3–C1; 84D1–2* + a point mutation at *Antp*	*Antp*
Df(3R)dsx^{D}+^{R1,2,8,10}	DUNCAN & KAUFMAN	*Df(3R)84D5–6; 84F16*	*dsx*

Revertants of Antp^{Ns}	Discoverer	Cytology	Sites exposed
T(3;4)Antp^{Ns}+^{R2}	DUNCAN & KAUFMAN	*T(3;4)84B1–3;102F*	*Scr, Antp, Msc, Hu*
T(Y;3)Antp^{Ns}+^{R3}	DUNCAN & KAUFMAN	*T(Y;3)84A4–B2;Y*	*Antp, Hu*
Antp^{Ns}+^{R8}	DUNCAN & KAUFMAN	normal	*Antp, Hu*
Antp^{Ns}+^{R11}	DENELL	normal	*Antp, Hu*
T(2;3)Antp^{Ns}+^{R13}	DUNCAN & KAUFMAN	*T(2;3)84A4–B2; 40–41*	*Antp, Hu*
T(Y;3)Antp^{Ns}+^{R19}	DUNCAN & KAUFMAN	*T(Y;3)84B1–3;Y*	*Antp, Hu*
In(3R)Antp^{Ns}+^{R25}	DENELL	breakpoints in heterochromatin + 84B1–2+85A	*Antp, Hu*
In(3R)Antp^{Ns}+^{R72}	DENELL	*In(3R)84B3;84D*	*Antp, Hu*

Males homozygous for certain combinations of chromosome 3 markers were treated with X rays (4000R) or ethyl methanesulfonate (0.0125 M) (LEWIS and BACHER 1968) and mated to females heterozygous for 2 different chromosome 3 balancers, *In(3LR)CxD* and *In(3LR)TM3, ri p sep bx^{34e} Sb e^{s} Ser*. Note that the *TM3,Sb* balancer is distinguished by the dominant bristle marker, Stubble. Male progeny bearing one treated chromosome 3 and the *TM3,Sb* homolog were collected and pair-mated to virgin females that were heterozygous for *TM3,Sb* and *Df(3R)Antp^{Ns}+^{R17}*, which is deficient for region 84B-D. The absence of an F_2 progeny class bearing wild-type bristles (*Sb*+) indicated the induction of a lethal mutation in the region

exposed by the deficiency. Mutations demonstrating less than 80% viability were considered to be subvital, and less than 20%, semi-lethal. Visible mutations were noted as well. For lethal mutations, balanced stocks were generated by crossing sibs heterozygous for the mutagenized chromosome and *TM3,Sb*. Each mutation was retested by crossing males from the balanced stocks to *Df(3R)Antp^{Ns+R17}/TM3,Sb* females.

Mutations were named by the following alpha-numeric code. A capital letter E or X indicates mutagenesis by EMS or X rays, respectively, and is followed by a lower-case letter designating the particular chromosome on which the lesion was induced: "a" (*Dfd p^{p}*), "b" (*Ki roe p^{p}*), "c" (*Ki p^{p}*), "d" (*p^{p} cu*), or "e" (*Ki p^{p} bx sr e^{s}*). These 2 letters are followed by the initial of the discoverer and finally, a number to identify the particular mutation by its order of discovery. Nine EMS-induced mutations, designated only by numbers, isolated by T. A. GRIGLIATTI at the University of British Columbia, also were incorporated into this analysis.

Visible mutations were designated in a manner reflecting their phenotype. (*roe* = roughened eye, *ril* = rickety legs and *mab* = malformed abdomen.

Overlapping deficiencies spanning 84B-D were utilized to establish preliminary localizations of the recovered mutations. Four revertants of double sex dominant (DUNCAN and KAUFMAN 1975) overlap the distal four bands of the region deficient in *Df(3R)Antp^{Ns+R17}*. *Df(3R)Scr* (SINCLAIR 1977) overlaps the screening deficiency in the vicinity of the homoeotic cluster. *Df(3R)Antp^{+R2}* is a spontaneous reversion of *Antp^{7sb}* (DUNCAN and KAUFMAN 1975). *Df(3R)Antp^{+R1P}* is a recombinant chromosome derived from *In(3R)Antp^{+R1}*, a spontaneous reversion of *Antp^{7sb}* (M. M. GREEN, personal communication). The parent chromosome, *In(3R)Antp^{+R1}*, contains a small inversion from 84B to D. Additionally, a part of the inverted material is transposed to section 87, distal to curled. *Df(3R)Antp^{+R1P}*, containing the proximal portion of the *In(3R)Antp^{+R1}* chromosome, was recovered as an exchange between the inversion in 84 and curled on a *p^{p} cu* homolog, resulting in a chromosome deficient for the material near and including the roughened eye locus, which is present in the transposition. The cytological limits of these deletions are described in Table 1 and presented in Figure 1. These deletions define 7 subregions within the 84B-D interval. Complementation relationships within groups of lesions defined by deficiency mapping were discerned by *inter se* crosses.

Thanks to the wealth of lesions already available in the ANT-C (DENELL 1973; DUNCAN and KAUFMAN 1975; KAUFMAN, LEWIS and WAKIMOTO 1980), a finer-grained approach was undertaken to describe the newly induced mutations mapping to this region. In addition to the preliminary deficiency analysis, new mutations were crossed to all of the extant homoeotic mutations of this proximal series, as well as to several induced revertants of the dominant homoeotic mutation, *Antp^{Ns}* (DENELL 1973; DUNCAN and KAUFMAN 1975).

Standard salivary gland polytene chromosome squashes of the X-ray-induced mutations were stained with lacto-aceto orcein and examined with phase contrast microscopy. Breakpoints were localized using the maps of LEFEVRE (1976).

The scanning electron micrographs (SEM) presented in this paper were prepared by gluing live flies to SEM stubs with silver conductive paint and observing them without fixation or coating.

RESULTS

Screen: Successful testcrosses involving 3,791 mutagenized third chromosomes yielded a total of 92 mutations, 16 of X-ray origin, the remainder induced with EMS. Nine mutations induced with EMS at the University of British Columbia were also studied. Altogether, 80 of the new lesions have been classified as recessive lethal and 12 as visible mutations. Many of the visibles, three of which are dominant, additionally demonstrate lowered viability in heterozygous combination with the screening deficiency. For a distribution of these mutations among the various genetic backgrounds used see Table 2.

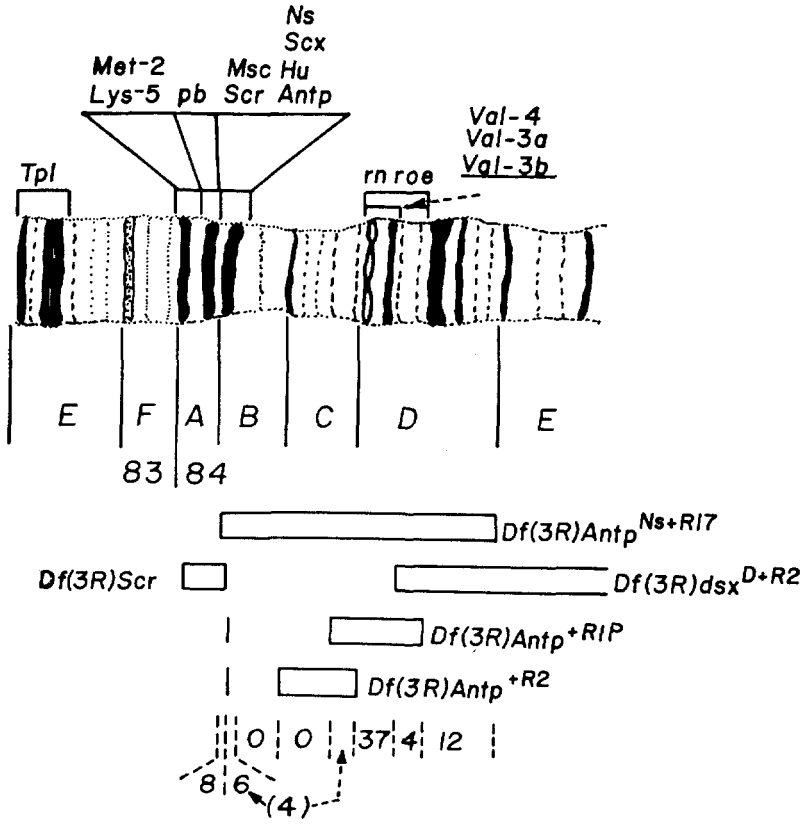

FIGURE 1.—Diagrammatic representation of polytene chromosome segments 84A-D. Cytology of this region is as in KAUFMAN, LEWIS and WAKIMOTO 1980. The open bars beneath the chromosome indicate the extent of the deletions utilized in this study. The dotted lines indicate the regions to which the recovered lethal and visible mutations are localized by deficiency mapping; the numbers indicate the number of mutations in each region. *Df(3R)dsx*$^{D+R2}$ has the same proximal breakpoint as the other three *dsx*$^{D+R}$ deletions used. *Tpl* = Triplolethal, *rn* = rotund, *roe* = roughened eye; the symbols of the homoeotic lesions are explained in Table 1. Met-2, Lys-5, Val-3a, Val-3b and Val-4 indicate the position of these tRNA's localized by *in situ* hybridization. (This information was obtained from S. HAYASHI and G. TENER.)

Deficiency mapping: The localization obtained by crossing each mutation to the eight overlapping deficiencies is shown in Figure 1. Descriptions of the groups thus defined follow.

Mutations that fail to complement Df(3R)dsx$^{D+R1,2,8,10}$: Twelve of the mutations fail to complement the four revertants of *dsx*^{D} and are thus localized to approximately four bands in the distal-most segment of section 84D. *Inter se* crosses yield the pattern shown in Figure 2. One allelic series contains seven members. Another pair of alleles, *EbD10* and *EbR24*, is involved with the development of male genitalia. *EbD10* was classified as a visible mutation due to the rotation of the male genitalia when in heterozygous combination with *Df(3R)Antp*$^{Ns+R17}$. *EbD10/EbR24* produces the same phenotype, although *EbR24* is lethal over the

TABLE 2

Distribution and description of mutations induced on marked third chromosomes

Chromosome	Mutagen	Desig-nation	Total scored	No. lethals	No. visibles	Phenotypes of visibles
Dfd p^p	X rays	Xa	666	8		
Ki roe p^p	X rays	Xb	766	8		
Ki roe p^p	EMS	Eb	991	27	3	*Ebmab*^{1} = malformed abdomen, ts* *Ebroe*^{en} = roughened eye enhancer, ts*
						EbD10 = rotated genitalia
Ki p^p	EMS	Ec	418	13	1	*Ecmab*^{2} = malformed abdomen
p^p *cu*	EMS	Ed	892	31	6	*Edroe*^{2} = rough eyes, *EdK5* = rough eyes, fine bristles *Edril* = rickety legs *EdK6*, *EdD8*, *EdR18* } Dominant reduced sex combs
Ki p^p *bx sr* e^s	EMS	Ee	58	5		
			3791	92	10	

* ts = temperature sensitive.

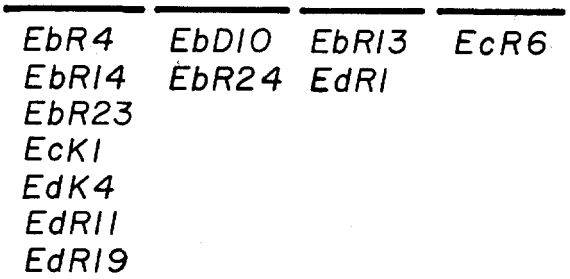

FIGURE 2.—Complementation map of the 12 mutations exposed by *Df(3R)dsx*$^{D+R1,2,8,10}$ and the screening deficiency. The order of these groups is arbitrary.

screening deficiency. Two other functional groups are defined by *inter se* crosses, one of one member and the other of two. The order of these groups as shown in Figure 2 is arbitrary.

Mutations that fail to complement Df(3R)Antp^{+R1P} *and* Df(3R)dsx$^{D+R1,2,8,10}$: Four recessive lethals are exposed by the region of overlap between the *dsx*^{D} deficiencies and *Df(3R)Antp*$^{+R1P}$. Their *inter se* complementation pattern, shown in Figure 3, can be interpreted as representing a single functional site.

Mutations that fail to complement Df(3R)Antp^{+R1P}: A large array of mutations has been recovered that fail to complement *Df(3R)Antp*$^{+R1P}$ and the screening deficiency. The former chromosome deletes the roughened eye (*roe*) and rotund (*rn*) loci, as well as a valine-tRNA site (DUNN *et al.* 1979). Twenty-six lesions localized to this region have been recovered and analyzed in this laboratory, along with nine others induced on a p^p *cu* chromosome and recovered over *Df(3R)Antp*$^{+R1P}$ at the University of British Columbia at Vancouver.

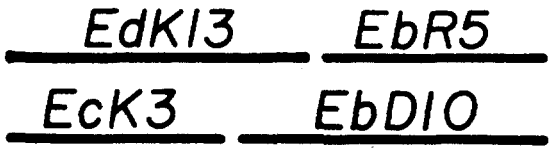

FIGURE 3.—Complementation map of the 4 mutations exposed by the dsx^{D+R} deficiencies and $Df(3R)Antp^{+R1P}$. The left-right orientation is arbitrary.

Pairwise crosses between all members of this group of mutations at 25° have revealed an intriguing organizational and functional complexity. The total complementation pattern is most simply interpreted as a circle, characterized by two somewhat overlapping groups of mutations designated "cluster A" and "cluster B" in Figure 4. The term "cluster" is used to denote functional, but not necessarily physical, overlap of the mutations. Three "spikes" (*XaD5*, *EeR1*,

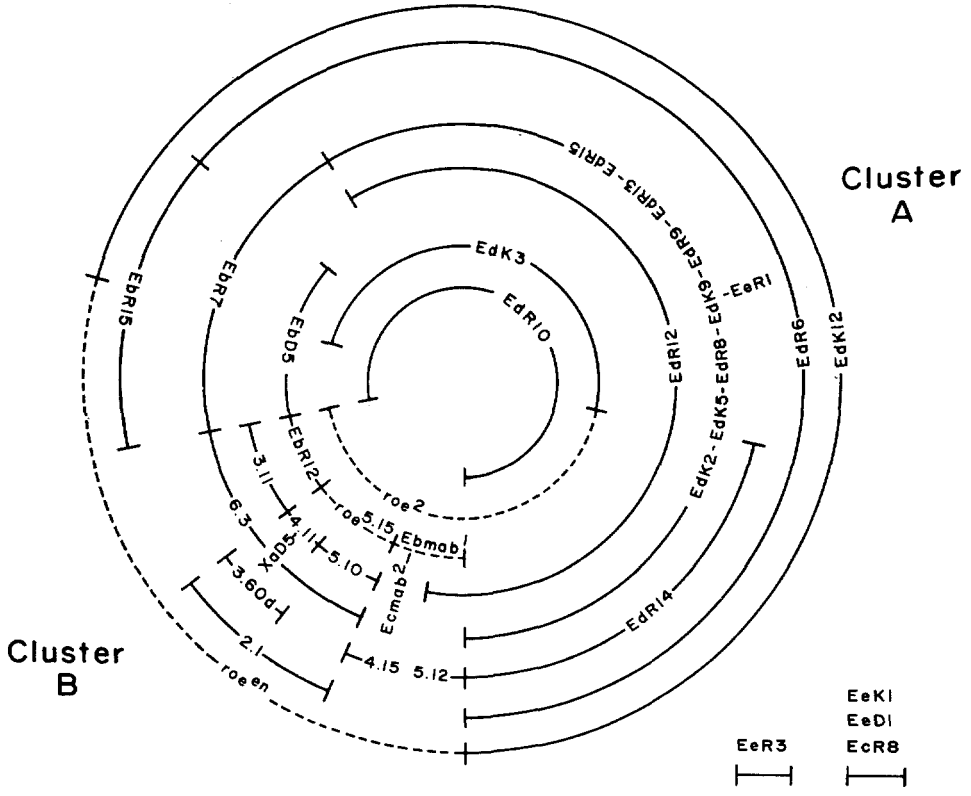

FIGURE 4.—Complementation map of lethal interactions at 25° of mutations exposed by $Df(3R)Antp^{+R1P}$. Solid lines indicate mutations that are lethal in combination with $Df(3R)Antp^{Ns+R17}$; dotted lines indicate mutations that produce a visible phenotype in combination with $Df(3R)Antp^{Ns+R17}$. For an explanation of the mutant symbols used in Table 2, see MATERIALS AND METHODS. Those mutations represented as perpendicular to the circle (*XaD5*, $Ecmab^2$, *EeR1*) fail to complement, in addition to the two deletions, only the mutations to which they are joined in the diagram. Two additional sites exposed by the deficiencies complement the circle.

Ecmab^{2}), mutations that fail to complement only one member of the circle, are indicated as well. Four other lesions define two additional sites, separate from each other and the circle. Each mutation involved in the circle is semi-lethal in heterozygous combination with at least one other member of the circle.

An additional peculiarity is evident in the asymmetric distribution of mutations of similar genetic background, although exceptions do exist. Cluster A is entirely composed of mutations isolated in Bloomington on a p^{p} *cu* chromosome. Cluster B contains two subgroups, one composed of three mutations induced in a *Ki roe* p^{p} chromosome (*EbD5*, *EbR7*, *EbR15*) and another containing the numbered mutations isolated in Vancouver on a p^{p} *cu* chromosome. The three spikes differ in genetic background from the areas from which they radiate. Specifically, *XaD5* and *EeR1* emanate from p^{p} *cu* backgrounds; *Ecmab*^{2} from a *Ki roe* p^{p} background. Of the six visible mutations, three, (*Ebmab*^{1}, *5.15*, *roe*^{en}) occur in cluster B, one (*EdK5*) in cluster A, one as a spike (*Ecmab*^{2}) and one (*roe*^{2}) encompasses both clusters. *EdK5*, *5.15*, *roe*^{en} and *roe*^{2} all confer a recessive, roughened-eye, phenotype at 25°. *Ebmab*^{1} and *Ecmab*^{2} each produce a recessive abdominal malformation. Additionally, the cluster A mutation, *EdK3*, although lethal at 25°, is fully viable and displays fine bristles at 18°. A dominant misfusion of posterior tergites is also associated with *roe*^{en} at 28°. All visible mutations are characterized by negative complementation; that is, a lesion exhibiting a visible phenotype when heterozygous with a deficiency dies when heterozygous with certain lethal members of the complex.

All but two (*roe*$^{5.15}$ and *roe*^{2}) of the visible mutations are temperature sensitive. Cluster A mutation *EdK5* and cluster B mutations *Ebmab*^{1} and *Ecmab*^{2} appear wild type at 18°, visibly mutant at 25° and lethal at 28°. *EdK5* has a pleiotropic phenotype, exhibiting fine bristles at 18°, roughened eyes and fine bristles at 25° and is lethal at 28°. The *roe*^{en} mutation in cluster B yields a visible phenotype at 25°, but is lethal at either temperature extreme.

One mutation within this group has permitted a tentative localization of the complex to between 84C1–5. *XaD5*, the only X-ray-induced lesion in the group, is a *2L-3R* translocation in which the *3R* breakpoint occurs in 84C just distal to 84C1 and the *2L* break in 21C.

Mutations that fail to complement Df(3R)Antp^{+R1P} *and* Df(3R)Antp^{+R2}: Four mutations, *XbD2*, *EbR27*, *EdR16* and *EdR17*, fall into this group. *Inter se* crosses define a single allelic series whose location is problematical. The deleted material of both deficiencies is distal to 84B1,2; however, both chromosomes also fail to complement the recessive lethality of *Antp*^{73b}. Since all four of these mutations complement *Df(3R)Scr*, it seems likely that this group resides in the distal deleted region. However, this conclusion is complicated by complementation results presented below.

Mutations that fail to complement Df(3R)Scr, Df(3R)Antp^{+R1P} *and* Df(3R)-Antp^{+R2}: *EcK5*, *EcR10*, *EbD5*, *EeR4* and *XbK4* comprise this group. The *XbK4* chromosome appears cytologically to involve two aberrations, one a pericentric inversion [*In(3LR)62B,98F*] of chromosome 3 and the other a 2;3 translocation [*T(2;3)36C,D; 84B1,2*]. One additional mutation, *EbR17*, differs from the above

five in that it complements *Df(3R)Antp*$^{+R2}$; however, *inter se* crosses of all six reveal only a single complementation unit.

Mutations that fail to complement Df(3R)Scr: Seven mutations are exposed only by this most proximal deficiency. *Inter se* crosses reveal two distinct complementation groups. One of the two includes *EbR11*, *XaK2*, *XaK5* and *XaK26*. The latter three mutations exist in chromosomes that appear to be cytologically normal.

The second group of mutations located within the overlap of *Df(3R)Scr* and *Df(3R)Antp*$^{Ns+R17}$ consists of *EdK6*, *EdD8*, *EdR18*. This group is characterized by reduction in the number of sex-comb teeth in mutant/+ males. The two deficiencies that localize these mutations also display this phenotype. Therefore, we have named this site the *Sex-Combs Reduced* (*Scr*) locus.

EdR18 appears to be a semi-lethal allele of the *Scr* locus. Surviving heterozygotes (*EdR18/EdD8*, *EdR18*/deficiency) are characterized by a severe reduction in number and sometimes absence of the sex-comb teeth. Those teeth that do appear are not rotated and are frequently thin and weakly pigmented (Figure 5). The chaetotaxy in general appears to be more consistent with that of a mesothoracic leg, *e.g.*, absence of transverse rows. We interpret these observations to indicate a homoeotic transformation of prothoracic to mesothoracic leg. A more quantitative analysis of this phenomenon appears in the companion paper (LEWIS *et al.* 1980).

An additional transformation observed in *EdR18/Df(3R)Scr* individuals affects the proboscis. The labial palps appear to be transformed into maxillary palps, with a concomitant, although variable, reduction in the number of pseudotracheal rows (Figure 5).

Mutations that complement all small deficiencies: Thirty-seven mutations that fail to complement *Df(3R)Antp*$^{Ns+R17}$ complement all seven overlapping deficiencies. Two hypotheses are suggested to explain the complementation behavior of these mutations. First, the screening deficiency may contain lesions elsewhere at an unusually mutable site(s). Second, this group could reside in the region exposed only by *Df(3R)Antp*$^{Ns+R17}$, but by none of the included deficiencies (Figure 1).

Inter se crosses among this group reveal one large, complex, complementation unit involving 34 of the 37 lesions. One of the 34, *Edril*, survives at low frequency in heterozygous combination with *Df(3R)Antp*$^{Ns+R17}$ to produce individuals characterized by a thickening of the metathoracic legs. Another member of this group, *EcR4*, originally defined as a lethal with *Df(3R)Antp*$^{Ns+R17}$, produces an identical leg phenotype in heterozygous combination with ten of the other lethals. Preliminary mapping experiments indicate that the rickety leg complementation group resides quite far from the site conferring the *Antp* phenotype and recessive lethality, and thus from the ANT-C. The other three complementing lethals map 1.5, 11 and 26 map units away from the ANT-C. It appears, therefore, that the first hypothesis is the more likely; these mutations will not be discussed further.

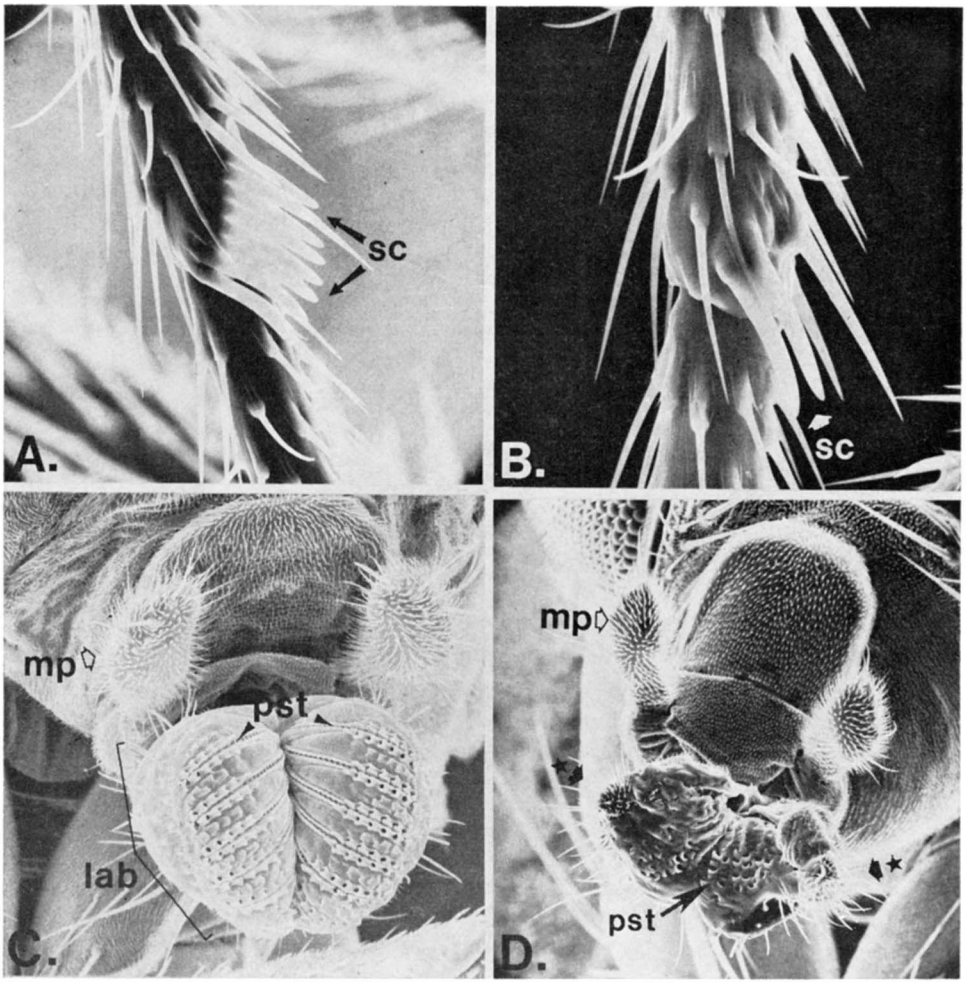

FIGURE 5.—SEM micrographs of normal and *EdR18* prothoracic sex combs and labial palps. (A) Normal Ore-R sex combs (sc) and basitarsus. (B) Basitarsus showing the reduction in number of sex comb teeth in *EdR18/EdD8* males. Also note the two remaining teeth are not rotated. (C) Normal Ore-R labial (lab) and maxillary (mp) palps. Note the pseudotracheal rows (pst) on the labial palps. (D) Labial palps of an *EdR18/Df(3R)Scr* male. The lateral aspect of the palps (star) is hairy and appears to be transformed into cuticle resembling maxillary palp.

Additional crosses to further delineate 84B1,2 mutations: Crosses to homoeotics: Relationships of the newly induced mutations mapping to 84B1,2 (above) to known homoeotic lesions resident in this region have been examined.

The group comprised of *EcK5*, *EcR10*, *EbD7*, *EeR4*, *XbK4* and *EbR17* fails to complement the recessive lethality of $Antp^{73b}$, $Antp^{50}$, $Antp^{B}$, $Antp^{R}$ and $Antp^{Scx}$. Individuals heterozygous for members of this group and the dominant homoeotic mutation Humeral (*Hu*) display a humeral phenotype as extreme as that of *Hu/Df(3R)Scr* (KAUFMAN, LEWIS and WAKIMOTO 1980).

The four members of the Sex-Combs Reduced locus fail to complement the

recessive lethality of *Msc*. *EdR18/Msc* flies demonstrate 25% viability and are characterized by the labial to maxillary palp transformation associated with other heterozygotes involving *EdR18*. Males of this class exhibit a drastic reduction in the number of sex-comb teeth beyond that seen in *Msc*/+ or *EdR18*/+ males. These observations place the *Msc*-associated lethality in this group.

The other group of mutations that fail to complement *Df(3R)Scr*, including *EbR11*, *XaK2*, *XaK5* and *XaK26*, complements all tested homoeotics (Figure 6).

The four mutations, *EbR27*, *EdR16*, *EdR17* and *XbD2*, that fail to complement $Df(3R)Antp^{+R1P}$ and $Df(3R)Antp^{+R2}$ also fail to complement the recessive lethality of $Antp^{73b}$. However, they complement the recessive lethalities of $Antp^{B}$, $Antp^{50}$ and $Antp^{R}$, as well as all other lethals in this group (Figure 6). Since the two deficiencies used to define this group are spontaneous revertants of a chromosome carrying $Antp^{73b}$, it is possible that these four new mutations define a lethal site on the parent $Antp^{73b}$ chromosome extraneous to the homoeotic lesion but still within the 84B–D interval. This hypothesis is consistent with the observation that the $Antp^{73b}$ chromosome is aberrant in section 84C–D, distal to the

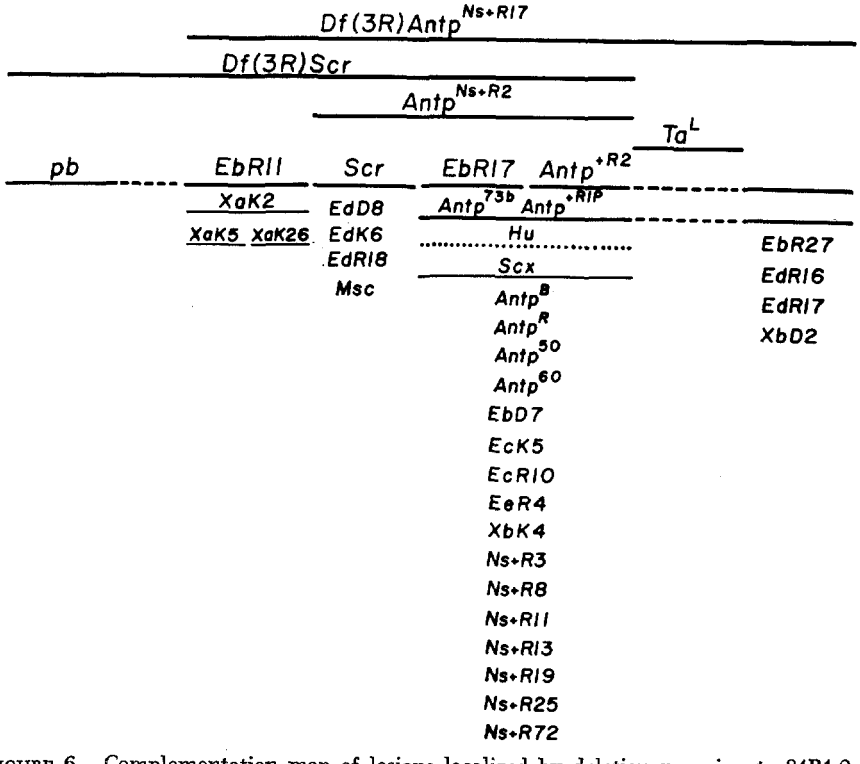

FIGURE 6.—Complementation map of lesions localized by deletion mapping to 84B1,2. The group headed by *EbR27* is probably not located in this interval, but more distal in 84C (see RESULTS). The left-right order of the *Scr* and *Antp* groups is known (KAUFMAN, LEWIS and WAKIMOTO 1980). The positions of the *EbR11* group is not known, except that it is unlikely to reside between *Scr* and *Antp*. These results have defined three lethal complementation groups in 84B1,2, two of which are associated with dominant homoeotic transformations.

84B1,2 site of *Antp* (T. HAZELRIGG, personal communication).

All 18 new mutations associated with 84B1,2 complement proboscipedia (*pb*) and the recessive lethality of Thickened Arista (Ta^2) (KAUFMAN, LEWIS and WAKIMOTO 1980).

Crosses to revertants of $Antp^{Ns}$: The 17 mutations possibly involved in the ANT-C have been crossed to eight revertants of the dominant homoeotic mutation, $Antp^{Ns}$ (Figure 6). The results of these crosses substantiate the complementation patterns established by the aforementioned crosses.

All eight revertants fail to complement the recessive lethality of the *EcR10-EcK5-EbD7-XbK4-EeR4-EbR17* allelic series and confirm their assignment to the *Antp-*$Antp^{Scx}$*-Hu* locus.

One of the $Antp^{Ns}$ revertants, the translocation $T(3;4)Antp^{Ns+R2}$, fails to complement *EdK6* and *EdD8* and is semi-lethal in combination with *EdR18*, an observation consistent with the failure of this revertant to complement *Msc* (DUNCAN and KAUFMAN 1975). Recall that *EdK6*, *EdD8*, *EdR18* and *Msc* comprise the *Scr* locus. Like the other $Antp^{Ns}$ revertants, $T(3;4)Antp^{Ns+R2}$ complements the recessive lethality of *EbR11*. Hence, the localization of the *Scr* group adjacent to the *Antp* locus, as was shown previously (KAUFMAN, LEWIS and WAKIMOTO 1980), is confirmed by the fact that both fail to complement $T(3;4)$-$Antp^{Ns+R2}$.

DISCUSSION

The recovery and localization of 13 new mutations to the 84B1,2 doublet have placed three clearly defined loci, at least two of which are homoeotic in nature, in the repertoire of mutations associated with this region of the chromosome. A more complete genetic and preliminary developmental analysis of the ANT-C will be presented in the accompanying paper.

The remainder of section 84B appears in cytological preparations to contain only one additional band. Apparently, we have not recovered any mutations that map in this region.

The overlap between $Df(3R)Antp^{+R1P}$ and the deficiencies obtained by reversion of the dominant mutation double sex^D involve at most one or two salivary bands. One functional site has been revealed by the four mutations mapping to this region.

Four bands in the distal part of section 84D are exposed by the double sex revertants, but not by $Df(3R)Antp^{+R1P}$. The 12 mutations recovered in this region are organized into four complementation groups.

A most interesting group of mutations, characterized by an intricate complementation pattern, temperature sensitivity and visible as well as lethal phenotypes, that fail to complement $Df(3R)Antp^{+R1P}$ has been recovered (Figure 4). The simplest complementation map, based on crosses performed at 25°, is a circle with three linear appendages. The mutations are distributed into two clusters: (1) cluster A composed of predominantly lethal alleles; and (2) cluster B, with three subsites involving two visible phenotypes, one conferring rough-

ened eyes and the other, a malformed abdomen. The lethal mutations demonstrate extensive semilethality, and the visible mutations are characterized by negative complementation and some temperature sensitivity. Additionally, the mutations are distributed in a nonrandom fashion with respect to genetic background.

Complementation maps deviating from the more common linear pattern, plus the phenomenon of negative complementation, have traditionally been interpreted as reflecting the interactions and conformations of protein subunit products. This has been substantiated by *in vitro* experiments using purified proteins from intragenically noncomplementing mutations (FINCHAM 1966). FINCHAM hypothesized that segments on a complementation map correspond to physical regions of the subunit product. Thus, the shape of the complementation map would reflect the physical proximities of the polypeptide regions distorted by each mutation. Nucleotide sequences far apart from one another on the chromosome might code for amino acid sequences that are close together in the final, folded protein product, and *vice versa.* CRICK and ORGEL (1964) attribute oddly shaped complementation maps to mutations resulting in misfolding of the protein product. Additionally, negative complementation observed among temperature-sensitive mutations of phage T4D has been interpreted in terms of a subunit interaction (BERNSTEIN, EDGAR and DENHARDT 1965).

It seems possible that the circular and negative complementation observed in this complex also describe a subunit interaction, with the temperature-sensitive alleles directing the synthesis of thermolabile variants. Genotypes of the variety "visible"/deficiency are viable, but visibly mutant, because the deficient chromosome does not direct synthesis of a product that might interfere in a destructive manner. However, "visible"/"lethal" combinations resulting in lethality may reflect such a destructive interaction.

Mutations conferring visible phenotypes similar to those associated with the circle—roughened eyes, malformed abdomens, fine bristles—occur quite frequently throughout the genome. Some are clearly implicated in protein synthesis, such as the 18S and 28S ribosomal genes coded for by the bobbed locus (*bb*, *1*–66.0) and the 5S RNA genes (PROCUNIER and DUNN 1978). Somewhat less clearly involved is the *X*-linked Abnormal-abdomen (A^{539}) gene and its modifiers, which, in addition to producing a phenotype remarkably similar to the *mab* alleles of this study, are believed to affect complexing abilities of aminoacyl tRNA synthetases (HILLMAN 1977). However, we realize that other mutations of this sort may not be involved in protein synthesis at all.

Nonetheless, on the basis of the circular and negative complementation patterns exhibited by this group of mutations, in conjunction with the nature of the visible phenotypes and their temperature sensitivity, our current working hypothesis is that the gene product(s) is a multimeric protein, possibly coded for by the two clusters, which may be involved in some manner with protein synthesis. However, a definitive answer to this question awaits a biochemical characterization of the mutations within this locus.

In summary, a mutagenesis and screening experiment designed to uncover mutations residing in the region of salivary bands 84B–D has revealed at least two genetically and functionally complex collections of complementation groups. Three lethal complementation units, two of which correspond to known homoeotic functions, have added 13 new genetic variants to the 84B1,2 region, thought to be the site of a collection of genes involved in the development of the head and thoracic segments. A second complex locus within the 84B–D interval involves 27 lethal and visible mutations that complement in the form of a biclustered circle.

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