A REHABILITATION OF THE GENETIC MAP OF THE 84B-D REGION IN *DROSOPHILA MELANOGASTER*

DOUGLAS R. CAVENER,* DEBORAH C. OTTESON† AND THOMAS C. KAUFMAN†,[1]

*Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235, and †Department of Biology, Indiana University, Bloomington, Indiana 47405

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ABSTRACT

A reanalysis of the 84B3 to 84D3,5 region of the polytene chromosomes of *Drosophila melanogaster* has led to the identification and localization of 16 genes. These genes include 11 vital loci, four genes exhibiting nonlethal visible mutant phenotypes and one gene encoding a nonessential enzyme. The identity of the gene products of two of the vital genes has been determined to be α-tubulin and glucose dehydrogenase (*Gld*). Three newly identified genes, *sticking* (*stk*), *half out* (*hat*) and *trapped* (*ted*), as well as *Gld* are required for eclosion. Among the nonessential genes are *roughened eye* (*roe*) and *ruffed eye* (*rue*), which affect eye texture. The roe phenotype is greatly enhanced by deletions that simultaneously remove *roe* and an unidentified locus in 84E. Mutations in another nonessential gene, *rotund* (*rn*), are characterized by pattern deletions of most adult appendages.

THE 84B-E region of the right arm of the third chromosome of *D. melanogaster* has received considerable attention during the past few years due to the presence of the Antennapedia complex (ANT-C) (KAUFMAN and ABBOTT 1984), two α-tubulin genes (RAFF 1984), the glucose dehydrogenase gene (CAVENER and MACINTYRE 1983) and *doublesex* (*dsx*) (DUNCAN and KAUFMAN 1975; BAKER and RIDGE 1980). A complementation analysis of this region was previously reported on by LEWIS *et al.* (1980). A revision of this analysis was necessitated by a number of new lesions recovered in this region and by some unfortunate errors in the initial analysis. We present here a revised complementation analysis of the region between the distal extent of the ANT-C (84B1,2) and the *Esterase-C* gene (*Est-C*) (84D3-5).

MATERIALS AND METHODS

All flies were reared on a diet of a cornmeal-molasses-yeast-agar medium at 25° ± 2. Most of the complementation crosses were performed independently by D.R.C. at Cornell University or Vanderbilt University and by D.O. and T.K. at Indiana University. A description of the various marker mutations and balancer chromosomes can be found in LINDSLEY and GRELL (1968).

[1] To whom correspondence should be addressed.

TABLE 1

Chromosomal deficiencies used in the mapping of sections 84B-D

Name	Cytology	Origin/reference
Df(3R)30c76	83C1,2-84B3,6	KEPPY and DENELL (1979)
Df(3R)Scr	84A1,2-84B1,2	KAUFMAN, LEWIS and WAKIMOTO (1980)
Df(3R)Antp$^{73b+RX1}$	83F5,6-84B1,2+84C1,2-84C6	HAZELRIGG and KAUFMAN (1983)
Df(3R)Antp$^{73b+RX2}$	83F5,6-84B1,2+84C1,2-84C6	HAZELRIGG and KAUFMAN (1983)
Df(3R)Scx^{W+RX2}	84B1,2-83C1,2	HAZELRIGG and KAUFMAN (1983)
Df(3R)Scx^{W+RX4}	84B1,2-84D3,4	HAZELRIGG and KAUFMAN (1983)
Df(3R)A41	84B1,2-84D1,2	ABBOTT (1985)
Df(3R)Antp^{+R1P}	84B1,2-84D1,4	DUNCAN and KAUFMAN (1975) KAUFMAN, LEWIS and WAKIMOTO (1980)
Df(3R)Antp^{Ns+R17}	84B1,2-84D14	DUNCAN and KAUFMAN (1975)
Df(3R)Hu^{+RX1}	84B1,2+84D5-84F8	HAZELRIGG and KAUFMAN (1983)
Df(3R)Win3	84A4,5-84B1,2	T. KAUFMAN[a]
Df(3R)dsx^{Mas+R2}	84C1,2-84E1	B. BAKER[a]
Df(3R)dsx$^{Mas+R10}$	84D3-84F1,2	B. BAKER[a]
Df(3R)dsx$^{Mas+R29}$	84C8,D1-84F6,7	B. BAKER[a]
Df(3R)dsx^{D+R2}	84D11-84F16;A1,2	DUNCAN and KAUFMAN (1975)
Df(3R)D6	84D2,3-84F13,16	I. DUNCAN[a]
Df(3R)D7	84D3,5-84F1,2	I. DUNCAN[a]

[a] Unpublished.

The procedures used to generate the X-ray and ethyl methanesulfonate (EMS) lesions described in this study were previously described (LEWIS *et al.* 1980; CAVENER and MACINTYRE 1983). The mutations described in this study were isolated from eight mutagenesis screens. The results from the first six screens have been published by LEWIS *et al.* (1980). Information obtained from two additional screens, one by D.R.C. and one by T.K. and D.O., are included in this report. All mutations described here are exposed by *Df(3R)Antp^{Ns+R17}* (84B1;84D11-12). This report examines a subset of the mutations that map within the breakpoints of *Df(3R)Antp^{Ns+R17}*, specifically those that map distal to the ANT-C (84B1,2) and proximal to the proximal breakpoint of *Df(3R)D7* (84D3-5).

Overlapping deficiencies (Table 1; Figure 1) were utilized to establish the approximate locations of the recovered mutations via complementation analyses. The deficiencies described in Table 1 define 11 segments (Figure 2) within 84B2,3-84D3,5. Mutations within each segment were crossed *inter se* in order to define individual complementation groups.

We define a complementation failure as the appearance of less than 5% of the expected number of the heterozygous complementation class. In all cases, except for mutations induced in the *p^{p} cu* chromosome, the Mendelian expectation was used inasmuch as the relative viability of the various segregating classes was nearly equal. Complementing mutations that were induced in the *p^{p} cu* chromosome have unusually low viability and a slow developmental rate. In order to accurately assess *inter se* crosses involving *p^{p} cu* mutants, progeny from complementation crosses were collected for 10 days after the beginning of eclosion. In addition, the Mendelian expectation was weighted by the relative viability of the *p^{p} cu* chromosome, determined in an independent experiment. The relative viability of the *p^{p} cu* chromosome was determined by computing the ratio of observed progeny arising from complementation crosses between mutations mapping in different deletion-defined segments.

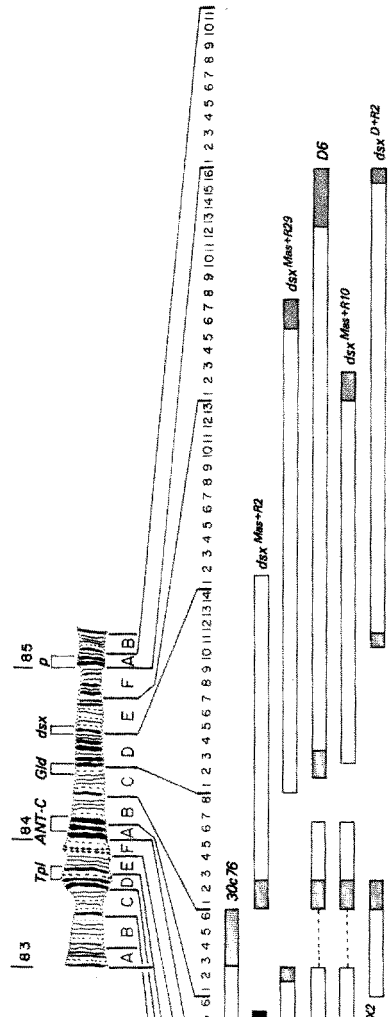

FIGURE 1.—Diagrammatic representation of the 83A-85B region of the right arm of the polytene chromosome map. The open bars below the chromosome show the extent of the deletion used in this study. The complete names and origin of these deletions are given in Table 1. The solid bar shows the extent of a duplication of material transposed to distal *3R* that is necessary to rescue *Df(3R)30c76*. This deletion includes the *Triplo-lethal* (*Tpl*) locus and therefore is normally lethal in heterozygotes. The dashed lines connecting two deficiency regions indicate that the chromosomal material between the two deleted areas is present. In all three cases these deletions were induced on inversion-bearing chromosomes, so that the deleted material is actually contiguous in the deficiency bearing chromosome. The shaded areas at the end of the deletion bars indicate ambiguity in the endpoints of the various deletions. The loci indicated above the chromosome are *Tpl*, *Triplo-lethal*; ANT-C, Antennapedia complex; *Gld*, *glucose dehydrogenase*; *dsx*, *doublesex*; *p*, *pink*.

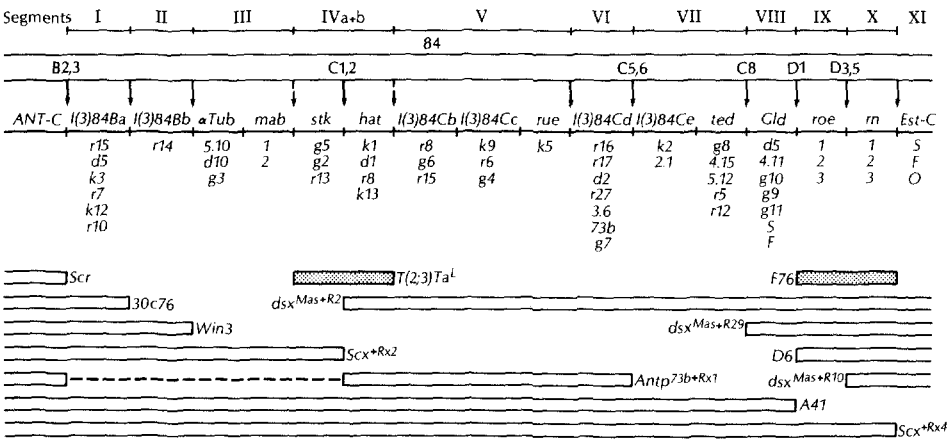

FIGURE 2.—Complementation map of the loci in the 84B3 through 84D5 region of the polytene chromosome map. The open bars at the bottom of the figure show the extent of the various deletions, the endpoints of which serve to demark the subsegments given at the top of the figure. These are numbered in order (I through XI) from proximal to distal on the chromosome. The arrows above and between the locus designations indicate the positions of breakpoints that allow unequivocal left-right orientation of the flanking genes. The B2,3; C1,2; C5,6; C8; D1 and D3,5 designations indicate the approximate cytological position of the indicated breakpoints. The allelic designation of the analyzed mutations are given below the line, the locus names above. The two shaded bars indicate the position of two mutations that are not visibly deleted in the polytene chromosomes and yet fail to complement two adjacent complementing loci. Loci that mutate to lethality but have not been well characterized are named by their location on the chromosome, *e.g.*, *l(3)84B* or *l(3)84C*. The lower-case letter following their designation is given alphabetically from proximal to distal within each lettered region. The abbreviations for the other loci correspond to the following names: ANT-C, Antennapedia complex; *α-Tub, alpha-Tubulin 84B*; *mab, malformed abdomen*; *stk, sticking*; *hat, half out*; *rue, ruffed eye*; *ted, trapped*; *Gld, glucose dehydrogenase*; *roe, roughened eye*; *rn, rotund*; *Est-C, Esterase-C*; Ta^l, *Thickened arista-Lethal*. The names and extents of the deletions are given in Table 1.

RESULTS

A deletion map of the 84B-D region is presented in Figure 1. We have subdivided the region between the ANT-C (84B1,2) and the proximal breakpoint of *Df(3R)D7* (84D3-5) into 11 segments ordered proximal to distal (Figure 2). These segments are delimited by deletions, except for segments III through V. Segments III through V are delimited by a combination of deletion breakpoints and a nonvariegating position effect of $T(2;3)Ta^L$. A description of the individual complementation groups within each segment is given below. The order of the complementation groups within each segment is unknown unless otherwise stated.

Segment I: The location of segment I is defined proximally by the distal breakpoint of *Df(3R)Scr* at 84B1,2 and distally by the distal extent of *Df(3R)30c76*. A single lethal complementation group, *l(3)84Ba*, which is comprised of six alleles, has been identified in this segment (Figure 2). Since the *Antennapedia* (*Antp*) locus is the next most proximal gene and this locus is resident in the substance of band 84B1,2, it is reasonable to conclude that

l(3)84Ba is located in the faintly banded region just distal to 84B1,2 perhaps in or adjacent to 84B3.

Segment II: The next most distal segment is delineated proximally by the distal end of *Df(3R)30c76* and distally by the distal end of *Df(3R)Win3*. Like segment I, there is only a single lethal complementation group in segment II, *l(3)84Bb* (Figure 2). There is, however, only a single mutant allele at this locus.

Segment III: The distal limit of *Df(3R)Win3* defines the proximal end of segment III. The distal end of this segment is proximal to the proximal limits of $Df(3R)dsx^{Mas+R2}$ and $Df(3R)Antp^{73b+RX1}$ (Figure 2). $Df(3R)Antp^{73b+RX2}$ shows the same complementation behavior as *RX1*, but is not shown in Figure 2. The distal limit of segment III is further refined by the complementation behavior of $T(2;3)Ta^{L}$. This translocation fails to complement two lethal loci apparently adjacent to the translocation breakpoint (see segment IVa and b below), but is viable in combination with the segment III mutation. There are two lethal complementation groups in this segment. The first is made up of a group of three lethals that are lesions in the major ubiquitous α-tubulin gene (K. A. MATTHEWS and T. C. KAUFMAN, unpublished results). The other locus has been called *malformed abdomen* (*mab*) due to the effects of the two mutant alleles on the development of cuticle in the sternites and tergites of the adult (LEWIS *et al.* 1980). The left-right order of the two loci is not known.

Segment IVa and b: This segment contains two lethal complementation groups, *sticking* (*stk*) and *half out* (*hat*). Heteroallelic combinations of *stk* mutations exhibit an undefined prepupariation effective lethal phase (ELP). Heteroallic combinations of *hat* alleles exhibit a late pupal ELP. The pharate adults appear to be morphologically normal. Indeed, many of these mutants partially eclose. The operculum opens and their heads and thoraces emerge. The inability of *hat* mutants to complete eclosion appears to be due to their legs being enmeshed in undegraded pupal cuticle. The partial eclosion phenotype displayed by *hat* mutants is also exhibited by $stk/T(2;3)Ta^{L}$ heterozygotes. Also, $hat/T(2;3)Ta^{L}$ heterozygotes partially eclose. Thus, the $T(2;3)Ta^{L}$ mutation fails to complement both *hat* and *stk* mutations and gives rise to the same lethal phenotype. The order of *stk* and *hat* is defined by three deficiencies: Scx^{W+RX2}, dsx^{Mas+R2}, and $Antp^{73b+RX1}$. The *stk* mutations fail to complement Scx^{W+RX2}, whereas *hat* mutations fail to complement dsx^{Mas+R2} and $Antp^{73b+RX1}$. Despite the complementation between Scx^{W+RX2} and *hat* and dsx^{Mas+R2} and *stk*, these two deletions fail to complement each other and are known to overlap by more than 10 kb (D. R. CAVENER, unpublished data). The Ta^{L} breakpoint occurs in this overlapping region. Taken together, these data indicate that $T(2;3)Ta^{L}$ may identify another vital locus in the *W+RX2/Mas+R2* overlap, but the failure of the translocation to complement *stk* and *hat* must be via a (nonvariegating) position effect. It is important to note that the Ta^{L} breakpoints in chromosomes *II* and *III* do not occur in heterochromatin.

Segment V: This segment is delimited by the distal position effect of $T(2;3)Ta^{L}$ and the distal breakpoint of $In(3R)Antp^{73b}$ (84C5,6) that mutates *l(3)84Cd* (see below). The two lethal complementation groups mapped to this segment [*l(3)84Cb* and *l(3)84Cc*] are characterized by a wide range of pupal

lethal phenotypes, depending on the specific heteroallelic combinations. The *g6* and *r15* alleles of *84Cb* complement each other, but both fail to complement *r8*. Our tentative interpretation of these results is that *g6/r15* heterozygotes display intragenic complementation. However, we cannot rule out more complicated interpretations. All three *84Cc* alleles fail to complement each other.

A single mutation affecting eye morphology also maps to segment V. This mutation (*k5*) exhibits roughened eyes and fine bristles at 25° and is lethal at 28° (LEWIS *et al.* 1980). We name this gene *ruffed eye* (*rue*).

Segment VI: A single lethal complementation group, *l(3)84Cd*, is delimited by the $In(3R)Antp^{73b}$ and the $Df(3R)Antp^{73b+RX1}$ breakpoints. $Antp^{73b+RX1}$ is a revertant of $Antp^{73b}$ and is associated with a deletion of the ANT-C and the 84C1,2-C5,6 region. DNA restriction analysis indicates that the distal breakpoints of $Antp^{73b}$ and $Antp^{73b+RX1}$ coincide (D. R. CAVENER, unpublished results). The distal breakpoint of $In(3R)Antp^{73b}$ also fails to complement six EMS-induced mutations that we denote as the *84Cd* complementation group. Mutations in *84Cd* exhibit an embryo-larval boundary ELP (R. LEWIS, personal communication).

Segment VII: The distal breakpoint of $In(3R)Antp^{73b}$, the distal extent of the $Df(3R)Antp^{73b+RX1}$ revertant in the normal chromosomal sequence and the proximal breakpoint of $Df(3R)dsx^{Mas+R29}$ delimit segment VII (Figure 2). This segment contains two complementation groups: *l(3)84Ce* and *trapped* (*ted*). The *2.1* mutation displays partial complementation with both the *k2* mutation and $Df(3)dsx^{Mas+R2}$. Heterozygotes of the genotype $2.1/dsx^{Mas+R2}$ which successfully eclose cannot fly, and walk abnormally. Interestingly, $k2/Scx^{W+RX4}$ heterozygotes exhibit the "half out" phenotype characteristic of *hat* mutants and stk/Ta^{L} heterozygotes. The *ted* mutants all exhibit an identical lethal phenotype: pharate adults attempt to eclose but cannot break the operculum seams. After a few days of fruitless effort, *ted* mutants die with their heads jammed into the anterior puparium. Excision of the anterior puparium of *ted* mutants before stage P15 will rescue virtually all such flies. Rescued adults are fully viable and fertile. This rescuable mutant phenotype is identical to the previously described *Gld* mutant phenotype (CAVENER and MACINTYRE 1983).

Segment VIII: This segment is defined by the proximal breakpoint of $dsx^{Mas+R29}$ (84C8-D1), and the distal breakpoint of *Df(3R)A41* (84D1,2) (Figure 2). The *Gld* gene is the only complementation group in segment VIII. The *Gld* mutant phenotype has been described previously (CAVENER and MACINTYRE 1983) and is identical to the *ted* mutant phenotype, as described above. $Df(3R)A41/Df(3R)dsx^{Mas+R29}$ heterozygotes also display a rescuable *Gld* mutant phenotype. DNA restriction analysis indicates that *A41* and $dsx^{Mas+R29}$ overlap by approximately 7 kb within the *Gld* transcription unit (D. R. CAVENER, unpublished results). The Gld^{d5} mutation is associated with a 2;3 translocation. Its third chromosome breakpoint maps to 84C8-D1 and is within 1 kb of the $dsx^{Mas+R29}$ breakpoint. These data indicate that *Gld* is located at 84C8-D1.

Segment IX: The proximal border of segment IX is defined by the proximal end of *Df(3R)D6* and the distal end of *Df(3R)A41*. Since both of these deletions have breakpoints at, or near, the proximal limit of region 84D (Table 1; Figure

2), segment IX is likely to be located in the 84D1,2 doublet. This conclusion is supported by the deletion that defines the distal limit of segment IX, *Df(3R)dsx*$^{Mas+R10}$. The proximal end of this deficiency is in 84D3 but does not include 84D1,2 (Table 1; Figure 1). There is a single complementation group in this segment, *roughened eye* (*roe*). All three mutant alleles produce a similar phenotype, which is shown in Figure 3. The preexisting *roe*^{1} allele produces a slight roughening of the eye due to the formation of irregular faceting. There is also a loss of interommatidial bristles at the posterior margin of the eye. The combination of *roe*^{1} and either certain deletions of the locus (*e.g.*, *Df(3R)Scx*$^{W+RX4}$) or the two newly derived alleles *roe*^{2} and *roe*^{3} gives a slight but detectable enhancement of the mutant phenotype (Figure 3C and D). Deletions that include *roe* and proceed distally along the chromosome to include region 84E (*e.g.*, *Df(3R)D6*) show a strong enhancement of the mutant phenotype. In these flies the eye is reduced in size, as well as being rough in texture (Figure 3E). The reduction in size is correlated with a distinct clumping of interommatidial bristles in the anterior portions of the eye (Figure 3E). This enhancement of mutant phenotype is apparently due to the deletion of a portion of the chromosome in 84E or possibly F concomitant with the deletion of *roe*. We have concluded, therefore, that a locus or loci distal to *roe* act in a dosage-sensitive manner to normally ameliorate the *roe*$^{-}$ defect; however, the nature of this interaction is not understood. Finally, the most extreme roe phenotype is observed in *Df(3R)Scx*$^{W+RX4}$/*Df(3R)D6* animals. These two deletions overlap at the *roe* locus and produce viable adults with the phenotype shown in Figure 3F. The eye is greatly reduced to about ¼ normal size, with a distinct clumping of the inter-ommatidial bristles at the anterior edge. Since these overlapping deletions are viable when heterozygous, it is unlikely that *roe* is a vital locus. However, it would appear that deletion of the locus causes a more severe disruption of eye development than do any of the identified point mutations. This observation, in turn, argues that they are all hypomorphic or leaky alleles. This conclusion is supported by the fact that all three alleles express a more severe phenotype in combination with a deletion than in any heteroallelic combination.

Segment X: The proximal limit of segment X is defined by the proximal extent of *Df(3R)dsx*$^{Mas+R10}$ and the distal limit by the distal breakpoint of *Df(3R)Scx*$^{W+RX4}$ (Figures 1 and 2). Since the cytological overlap of these two deletions is at 84D3 (Table 1), this would appear to be the site of the *rotund* (*rn*) locus, the only identified gene in segment X. Indeed, heterozygous individuals of the genotype *Df(3R)Scx*$^{W+RX4}$/*Df(3R)dsx*$^{Mas+R10}$ are viable and have a rotund phenotype. Therefore, like *roe*, *rn* is apparently not a vital locus. This conclusion is further supported by the survival of *Df(3R)Scx*$^{W+RX4}$/*Df(3R)D6* individuals which express both a roe and rn phenotype. The 84D3 location of *rn* is further supported by the fact that *rn*^{1} is associated with a translocation and inversion, one breakpoint of which is in 84D3 (Lewis *et al.* 1980). The *roe* and *rn* loci would appear to lie in close proximity at the proximal edge of 84D, based on the effects of an EMS-induced mutation *F76*. This chromosome was originally recovered because it carries a mutation at the *Sex Combs Reduced*

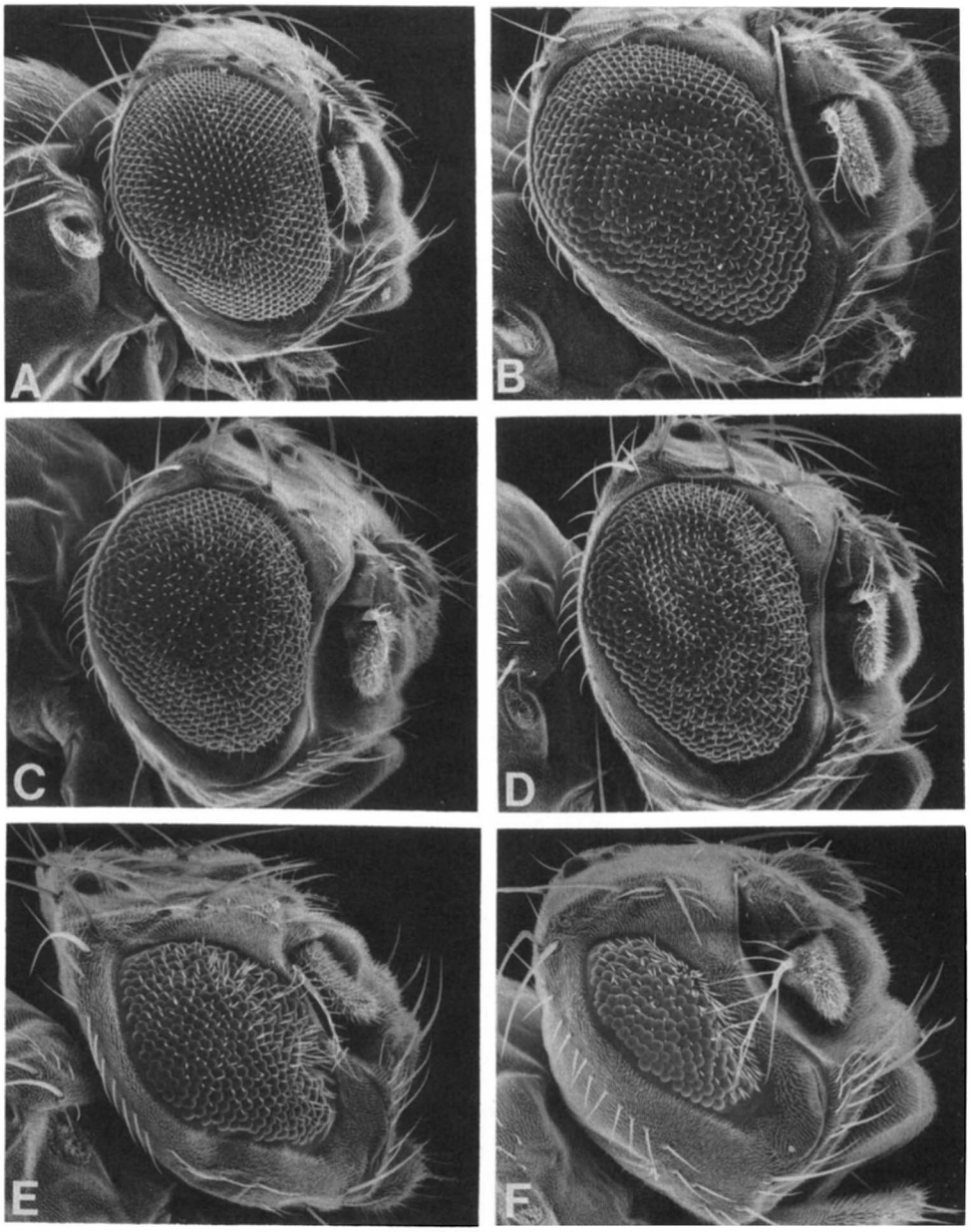

FIGURE 3.—Scanning electron micrographs showing the roughened eye (roe) phenotype. A, Oregon-R wild-type; note the regular array of interommatidial bristles. B, roe^1/roe^1. The interommatidial bristles are missing from the posterior portion of the eye, and the facets are irregularly formed in the center of the eye. C, $roe^1/Df(3R)Scx^{W+RX4}$; the eye is slightly more rough than in B but otherwise similar in phenotype. D, roe^1/roe^2. Animals of this genotype have an eye which is more rough than in B or C due to an increased disruption of facets in the central portion of the eye. E, $roe^1/Df(3R)D6$. The eye is reduced in size, and the interommatidial bristles are clustered in the anterior and absent in the posterior portion. This genotype shows the effects of deletion of the enhancer of *roe* in 84E-F on the roe phenotype. F, $Df(3R)Scx^{W+RX4}/Df(3R)D6$. These two deletions overlap at the *roe* and *rn* loci (see Figure 2) and produce the most extreme roe phenotype. The eye is greatly reduced, and the interommatidial bristles are entirely missing posteriorly and clumped at the anterior edge of the eye. Magnification: A and D, ×83; B and E, × 103; C, ×77; F, × 105.

(*Scr*) locus of the ANT-C. Subsequent genetic testing has revealed that this chromosome also carries a lesion that fails to complement the *roe* and *rn* mutations. Inspection of the polytene chromosomes of *F76/+* individuals has not revealed any striking chromosomal anomaly, *i.e.*, a visible deletion. Therefore, if the *F76* lesion is associated with a deletion, it apparently falls below the resolution of the light microscope.

Various aspects of the *rn* mutant phenotype are shown in Figure 4. The legs are shortened by the fusion of the tarsal segments (Figure 4A and B) and the apparent deletion of some tarsal cuticle. This deletion of structure can be seen more clearly by the effects of *rn* on the antennae. The third antennal segment is reduced in size, and the fourth antennal segment is missing (Figure 4C and D). The wing blade is also truncated by the absence of structures between the wing hinge and the more distal portions of the wing blade (Figure 4E and F). The reduction and deletion of structure in all of these appendages occurs in roughly the same proximal distal position. It would appear, therefore, that rn^+ functions in some aspect of the specification of structural elements in a similar region of a number of imaginal discs. In this regard it is similar to the *decapentaplegic* (*dpp*) locus (SPENCER, HOFFMAN and GELBART 1982). However, unlike *dpp*, *rn* does not appear to play a role in embryonic development, because a deletion of the locus has no apparent indispensable effect on embryogenesis.

Segment XI: The distal breakpoint of $Df(3R)Scx^{W+RX2}$ (Figure 2) and the proximal breakpoint of *Df(3R)D7* (Figure 1) delimit the XI segment. To date, no lethal mutations have been mapped in this region. Using standard electrophoretic analysis, we have found that the *Esterase-C* (*Est-C*) gene maps here. ONISHI and VOELKER (1982) had previously mapped *Est-C* to 84B2-D9. Our results delimit *Est-C* to a smaller region, 84D3-5. Putative *Est-C* null mutations are viable, consistent with our failure to identify lethal mutations in this segment.

DISCUSSION

We believe that nearly all of the genes within the 84B2,3-84D3,5 region have been identified. The 16 genes found include 11 vital genes, four genes that exhibit nonlethal but visible mutant phenotypes, and one gene identified strictly by electrophoretic analysis. This region contains 15–18 chromomeres. Thus, the number of chromomeres and the number of genes are approximately equal. Although the relationship of chromomeres and genes is still unknown, we note that a number of other studies in Drosophila have also indicated a one-to-one correspondence between these two parameters (for a review, see LEFEVRE 1974). However, inasmuch as two of the genes described here are represented by single mutations, it would not be surprising to find that we have failed to detect one or two genes in this region.

A previous report on this genetic region (LEWIS *et al.* 1980) postulated the presence of a complex circular complementation group composed of mutations that we now claim are members of several independent complementation groups. The previous analysis was largely based on a series of mutations in-

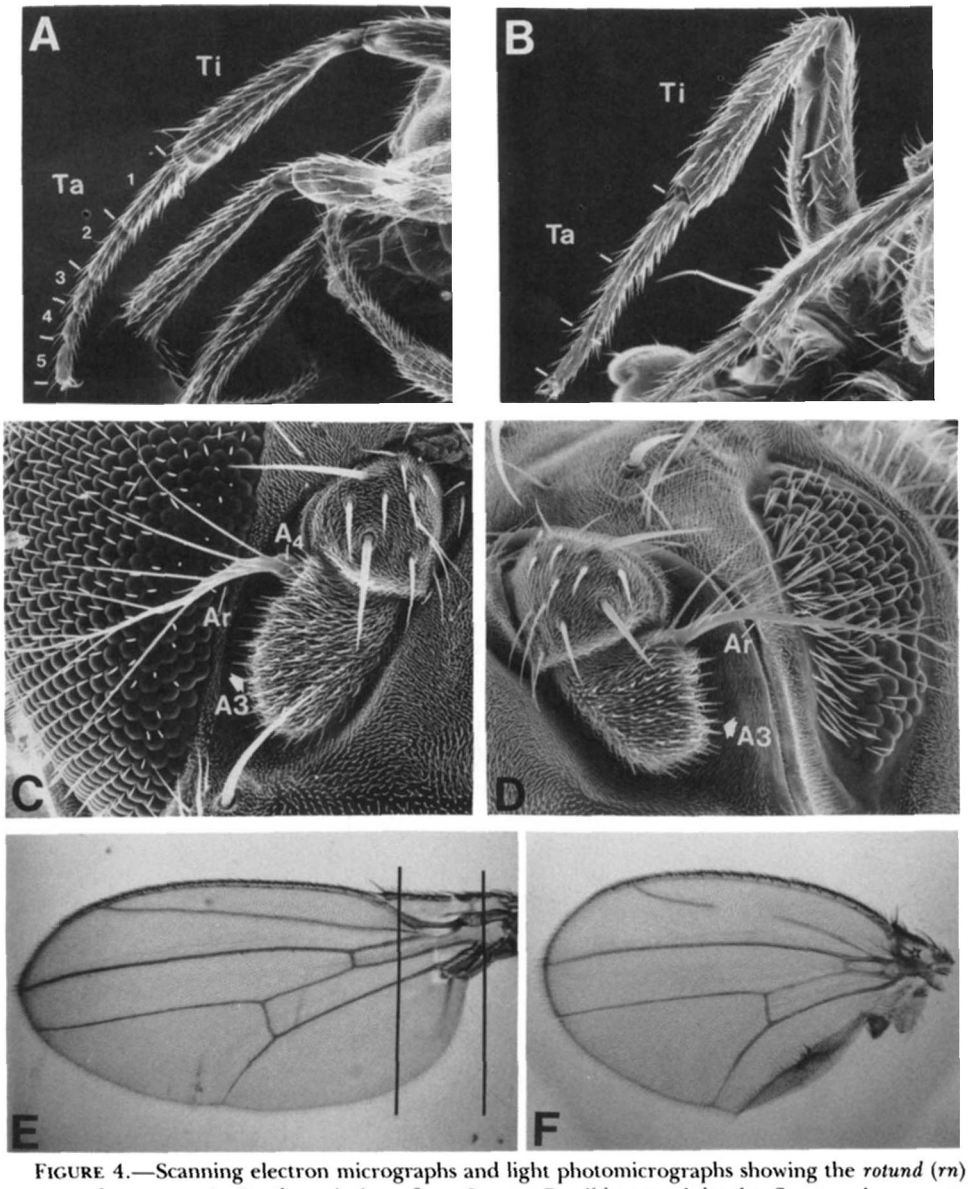

FIGURE 4.—Scanning electron micrographs and light photomicrographs showing the *rotund* (*rn*) mutant phenotype. A, Prothoracic leg of an Oregon-R wild-type adult; the five tarsal segments (Ta) are demarked by white lines and are numbered proximal to distal (Ti = tibia). B, Leg of a *Df(3R)Scx*$^{W+RX4}$/*Df(3R)D6*, *rn*$^{-}$ individual. Note the absence of tarsal segmentation and shortening of the tarsal region of the leg. C, Antenna of an Oregon-R wild-type. A3, third antennal segment; A4, fourth antennal segment; Ar, arista. D, Antenna of a *Df(3R)Scx*$^{W+RX4}$/*Df(3R)D6*, *rn*$^{-}$ individual. Note the reduction in size of the third antennal segment and the absence of A4. The missing and reduced portion of the antenna are serially homologous to those similarly affected in the leg. E, Wing of an Oregon-R wild-type individual. The black lines indicate the general area of the wing blade that is reduced or deleted in *rn* mutant animals. F, Wing of an *rn*$^{-}$ animal of the same genotype as B and D. Note the absence of the proximal portion of wing vein L1 (star) and the foreshortening of the proximal wing. The gap in the wing vein L2 is caused by the *radius incompletus* (*ri*) marker carried on the two deletion chromosomes; this effect is not caused by the *rn* deficiency. Magnification: A, ×60; B, ×94; C, ×165; D, ×182.

duced in a p^p *cu* strain. Unfortunately, this strain has relatively low viability and a slow developmental rate. The circular complementation group was based on the observation of semilethal complementation. The addition of several mutations induced in other strains, and the recovery of several new deletions, led us to repeat the complementation tests for this entire region. These experiments unequivocally indicated that a complex circular complementation group does not exist in this region as previously described.

Among the 11 vital genes in this region there are four that are required for eclosion: *stk*, *hat*, *ted* and *Gld*. *Gld* and *ted* exhibit identical eclosion mutant phenotypes. However, complementation crosses between *Gld* and *ted* do not indicate any genetic interactions. It is possible that the *Gld* and *ted* gene products lie in the same metabolic pathway and, thus, yield a similar mutant phenotype when absent. Unfortunately, the metabolic and physiological roles of the GLD enzyme are unknown in eukaryotes. Attempts to identify a metabolically related enzymatic function for the *ted* gene product have been unsuccessful (D. R. CAVENER, unpublished data). Apparently, the major developmental role of *Gld* and *ted* is to modify the puparium case in order to render it brittle and weak for easy escape during eclosion. Pressure applied to the operculum seams of stages P5–P15 wild-type pupae easily breaks the seams (simulating part of the eclosion process). In contrast, *Gld* and *ted* mutants display tough flexible puparium cases.

The *stk* and *hat* mutations are more complex genetically, in that they both fail to complement $T(2;3)Ta^L$ but complement each other. In addition, *stk* only exhibits an anomalous eclosion phenotype when heterozygous with Ta^L. Ta^L also fails to complement two deletions, Scx^{W+RX2} and dsx^{Mas+R2}, which fail to complement *stk* and *hat* mutations, respectively. The $T(2;3)Ta^L$ breakpoint has been mapped to a position within the ca. 10-kb region deleted in both Scx^{W+RX2} and dsx^{Mas+R2}. A position effect inactivation of the *stk* and *hat* genes by Ta^L is the only hypothesis that we believe is consistent with the genetic and molecular data. An additional complication in this region is the finding that Scx^{W+RX2} and dsx^{Mas+R2} fail to complement each other. Despite eight mutagenesis screens in this region, no EMS-induced lethal mutations have been found to map to the overlapping region of these two deletions. We conclude that (1) with the possible exception of Ta^L, we have simply failed to isolate mutations in a gene in this region; (2) the two deletion chromosomes fail to complement due to second site mutations; or (3) the combination of the two deletions inactivate *stk* and/or *hat* in a similar fashion to the $T(2;3)Ta^L$ breakpoint.

Incomplete apolysis of the pupal cuticle from the adult cuticle appears to be the developmental defect associated with the partial eclosion phenotype exhibited by *hat* mutants and stk/Ta^L heterozygotes. This defect is most apparent on the legs, where undegraded pupal cuticle enshrouds these structures and is also affixed to the puparium. Undegraded pupal cuticle is observed in vacated puparia of wild-type flies. Therefore, we do not know whether the presence of this cuticle surrounding the legs is the cause or an effect of the failure of eclosion in these mutants.

It is unlikely that the thickened aristae phenotype of $T(2;3)Ta^L$ is a direct

result of the breakpoint at 84C1,2. KAUFMAN, LEWIS and WAKIMOTO (1980) have shown that another *Ta*-like mutation maps to the ANT-C in 84B1,2. Furthermore, the thickened aristae phenotype of $T(2;3)Ta^L$ is enhanced in combination with deletions of the ANT-C. We speculate that $T(2;3)Ta^L$ is actually a complex aberration composed of an undefined lesion in 84B1,2 as well as the obvious translocation between the second and third chromosomes.

Two genes (*rue* and *roe*) mapping in the 84C-D region disrupt the normally smooth contour of the eyes when mutated. Over 50 genes affecting eye texture have been described in *D. melanogaster* (BRYANT and MURNIK 1980). In addition, many *Minutes* have a rough eye texture (LINDSLEY and GRELL 1968). It has been speculated that *Minutes* encoded gene products required for protein synthesis (*e.g.*, see RITOSSA, ATWOOD and SPIEGELMAN 1966). Indeed, KONGSUWAN and co-workers (1985) have demonstrated that *Minute(3)99D* encodes ribosomal protein 49.

A $tRNA^{Gly}$ gene has been mapped to 84C (HAYASHI *et al.* 1980), a light banded region including *rue*. Additionally, a $tRNA^{Val3b}$ gene has been mapped to the proximal end of 84D, which includes *roe*. Therefore, we suggest that *rue* and *roe* mutations may possibly represent lesions in these two tRNA loci. Since these regions of the genome have now been cloned, it will be possible to directly test this suggestion by mapping the tRNA genes relative to the *roe* and *rue* loci and/or by *P* element-mediated germline transformation of the normal tRNA sequences into mutant backgrounds.

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