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MOLECULAR GENETICS OF THE *ROSY-ACE* REGION OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

Three hundred and fifteen kilobases of DNA from the *rosy-Ace* region on chromosome *3R* of *D. melanogaster* have previously been cloned and extensively characterized. We describe the isolation of nine new deficiency mutants that break within the 315-kb interval. The position of these breakpoints on the DNA map was determined by *in situ* and Southern hybridization. Further, we more precisely mapped the breakpoints of several deletions previously analyzed. The results permit us to delimit sequences essential to the known complementation groups in the region within approximately 20 kb in most cases. However, one gene, *B16-1*, is shown to contain essential sequences that span about 50 kb. Also, we demonstrate by overlapping deficiencies that a 45-kb DNA segment from the region, which includes one known complementation group, allows limited survival when deleted.

WE are combining molecular and genetic approaches to dissect a small region of the *Drosophila melanogaster* chromosome. Our aims are to examine the chromosomal distribution of functional genes, both those which are essential for viability and those which apparently are not, and to define the extent of nonessential DNA sequences.

The *rosy-Ace* interval is at 87D,E on the right arm of the third chromosome. HILLIKER *et al.* (1980) have saturated the region for recessive lethal mutations and have arranged the genetic complementation groups they constitute on a cytogenetic map, using a staggered series of deficiencies. We have cloned a 315-kb segment of the region by chromosomal walking (BENDER, SPIERER and HOGNESS 1983) and have mapped the 16 existing deficiency breaks that fall within the cloned interval, thus locating 12 complementation groups on the DNA map (SPIERER *et al.* 1983). Transcription maps were established at different times in development (HALL, MASON and SPIERER 1983) and in different specialized tissues (BOSSY, HALL and SPIERER 1984). We thus were able to

identify a total of 43 different transcripts, over three times more than the number of known complementation groups.

It has become important now to determine more precisely the boundaries of genes in the region. As well as being necessary for the eventual definition of which transcript is the product of each known gene, this determination should also serve to define DNA sequences not involved in any of the essential complementation groups. To this end we have generated and mapped nine new deficiencies that enter the region from both directions, and we also have mapped the breakpoints of some former deficiencies with greater precision. We now are able to localize ten of the 12 complementation groups to DNA segments of about 20 kb in length. Unexpectedly, one gene, *B16-1*, is revealed to be very large, covering a minimum of about 50 kb of sequence. The new deficiencies also delimit a region of about 45 kb, including one complementation group, which can be deleted and still yield a few viable flies (6%).

MATERIALS AND METHODS

Drosophila strains: Drosophila cultures were maintained on standard yeast, cornmeal, sucrose and agar medium. The mutant and balancer stocks are described in LINDSLEY and GRELL (1968) and HILLIKER *et al.* (1980) or are listed in Table 1.

Generation, screening and genetic mapping of deficiencies: In two different experiments, several new deletions with breakpoints in the *rosy-Ace* region were isolated. In the first screen (experiment 1) *In(3R)Na/Sb* males were collected and irradiated with X rays (4000 rad) and then were mated *en masse* to *cu kar/cu kar* virgin females (*kar* is located proximally to *rosy* and *Ace*). The progeny was checked for karmoisin flies, which were selected and used to establish stocks with either *MKRS* (in the case of *In(3R)Na* bearing flies) or *TM2* (in the case of *Sb* flies) balancer chromosomes. Each of the stocks were crossed to $Df(3R)ry^{619}/MKRS$ flies ($Df(3R)ry^{619}$ uncovers the 315-kb *rosy-Ace* interval). All noncomplementing mutants were analyzed individually by mating them to different point mutations included in the *rosy-Ace* region. From about 80,000 F_1 progeny, six new deletions (designated *N*) were recovered, with breakpoints in the region between 87D4,5 and 87E4,5.

In the second experiment we chose a marker mutation distal to *rosy* and *Ace*. ISING and BLOCK (1981) described a new insertion site of a movable DNA sequence (*TE*) carrying w^+ and rst^+ genes. The large transposing element, *TE39*, has its insertion at 88A. Males with *w/Y; TE39/TM3 Sb Ser* genotype were collected and irradiated with X rays (4000 rad) and then were mated *en masse* to virgin females with a genotype *w spl/w spl; Sb/Ser*. In the F_1 progeny, all the flies carrying the transposon have wild-type eye color, but in cases where the w^+ gene is mutated or deleted, the eyes will be white. The white-eyed F_1 flies were selected and crossed to flies with $Df(3R)kar^{SZ11}/TM3$ (the $Df(3R)kar^{SZ11}$ deletion uncovers the majority of the 315-kb walk). Only crosses in which deletion-bearing chromosomes were lethal over $Df(3R)kar^{SZ11}$ were used to set up stocks. The established stocks were crossed to selected lethals of the *rosy-Ace* region to determine the genetic positions of the newly induced deletions. Out of 400,000 F_1 progeny, only three deficiencies (designated *GE*) had a breakpoint in the region of interest.

Recombinant DNA: All recombinant phages, together with coordinates on the DNA map of the walk, are as described by BENDER, SPIERER and HOGNESS (1983).

***In situ* hybridization:** Procedures for *in situ* hybridization to polytene chromosomes have been described (SPIERER *et al.* 1983).

Southern hybridization analysis of genomic DNA: Genomic DNA was prepared from adult flies essentially as described by BINGHAM, LEVIS and RUBIN (1981). Etherized flies (up to 500) were frozen in liquid nitrogen and were ground with a mortar and pestle. The frozen powder was mixed with 10 ml of nuclear isolation buffer

(10 mM Tris-HCl, pH 7.4, 60 mM NaCl, 10 mM EDTA, 0.15 mM spermidine, 0.15 mM spermine, 0.5% Triton X-100) and was suspended with two to three strokes in a Dounce homogenizer. Large particles were removed by spinning for 30 sec at 2000 rpm. The supernatant was then centrifuged for 7 min at 7000 rpm in a Sorvall RC-5B centrifuge. The pellet (a crude nuclear preparation) was resuspended in 3.6 ml of nuclear isolation buffer, to which 0.9 ml of 10% Sarkosyl was added and stirred in gently with a glass rod. The extract was mixed with 4.275 g CsCl and 0.45 ml ethidium bromide (10 mg/ml) and was centrifuged for 24 h at 37,000 rpm in a Beckman Ti50 rotor. The heavily stained DNA band was collected, the ethidium bromide was removed by three to four extractions with isoamyl alcohol and the DNA was precipitated with 2.5 volumes of ethanol. Electrophoresis of DNA, Southern transfer and hybridization, and preparation of nick-translated probes were performed as described previously (HALL, MASON and SPIERER 1983). Single restriction fragments from recombinant phages were prepared by cutting the band from a gel and then eluting the DNA electrophoretically in a dialysis bag. DNA was recovered from the buffer inside the dialysis bag by passing the contents over a small column of DE52 and eluting with 0.5 M NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA.

RESULTS

Generation of new deficiencies and genetic mapping: Fourteen deficiencies having breakpoints within our chromosomal walk have already been mapped by *in situ* hybridization and, in three cases, by Southern hybridization to genomic DNA (SPIERER *et al.* 1983). The 16 breakpoints allowed preliminary localization of 12 characterized complementation groups within the walk. To further delineate essential sequences and improve the resolution of the gene mapping, we undertook two further mutagenesis experiments.

Experiment 1 was designed to produce deficiencies entering the walk from its proximal end, whereas deficiencies from experiment 2 should enter the walk from its distal end. In experiment 1, after X-ray irradiation, we selected flies by the loss of the proximal eye-color marker *kar*. Six new deficiencies were recovered with breaks between 87D4,5 and 87E4,5; they are listed in Table 1 and are designated *N*. In experiment 2 we selected for the loss of a copy of the *white* gene carried on a transposing element inserted at 88A (*TE39;* ISING and BLOCK 1981). Three deletions, designated *GE*, were subsequently found to break within the region of interest (Table 1). Experimental procedures are described in MATERIALS AND METHODS.

The nine new deficiency-bearing stocks were tested for complementation with representative alleles of the different complementation groups in the *rosy-Ace* region to determine the genetic position of their breakpoints. The results are summarized in Table 1.

Localization of breakpoints on the DNA map: *In situ* hybridization was used in initial mapping of the deficiency breakpoints, as in our previous analysis (SPIERER *et al.* 1983). Selected recombinant phages from the walk were hybridized to salivary gland chromosomes bearing the deficiencies over a balancer chromosome. Failure to hybridize to the deficiency-bearing chromosome indicated that the DNA segment in question had been deleted. The experiment was repeated with appropriate recombinant phages until the breakpoint could be located between two adjacent DNA segments, of which one hybridized to the deficiency chromosome and the other failed to hybridize.

TABLE 1

Deficiencies with breakpoints in the walk

Deficiency	Source	Cytology of deficiency	Complementation								
			mes1A	*mes4B*	*pic*	*S8*	*B16-1*	*C9a*	*Ace*	*G7*	*m32*
Df(3R)N40	Experiment 1		–	–	–	–	–	–	+	+	+
Df(3R)N42	Experiment 1		–	–	–	–	–	–	–	+	+
Df(3R)N63	Experiment 1		–	–	–	–	–	–	–	–	+
Df(3R)N69	Experiment 1		–	–	–	–	–	+	+	+	+
Df(3R)N74	Experiment 1		–	–	–	+	+	+	+	+	+
Df(3R)N78	Experiment 1		–	–	–	–	–	–	–	+	+
Df(3R)GE26	Experiment 2		+	+	+	+	+	–	–	–	–
Df(3R)GE41	Experiment 2		+	+	+	+	+	+	+	–	–
Df(3R)GE99	Experiment 2		+	+	+	+	–	–	–	–	–
Df(3R)kar^{SZ11}	Gausz[a]	87C7-8; 87E5-6	–	–	–	–	–	–	–	–	+
Df(3R)kar^{lG27}	Hilliker[b]	87B3-5; 87D6-12[e]	–	–	+	+	+	+	+	+	+
Df(3R)lC4a	Hilliker[b]	87E5-7; 87E11-F1	–	–	–	–	–	–	–	–	–
Df(3R)l26c	Hilliker[b]	87E1-2; 87F11-12	+	+	+	+	–	–	–	–	–
Df(3R)l26d	Hilliker[b]	87D11-13; 87E3-5	+	+	–	–	–	–	–	–	–
Df(3R)ry^{36}	Hilliker[b]	Not visible	–	–	+	+	+	+	+	+	+
Df(3R)ry^{75}	Hilliker[b]	87D1-2; 87D14-E1	–	–	–	+	+	+	+	+	+
Df(3R)ry^{81}	Hilliker[b]	87C1-3; 87D14-E2	–	–	–	+	+	+	+	+	+
Df(3R)ry^{614}	Hilliker[b]	87D2-4; 87D11-14	–	–	–	+	+	+	+	+	+
Df(3R)ry^{619}	Hilliker[b]	87D7-9; 87E12-F1	–	–	–	–	–	–	–	–	–
Df(3R)ry^{1301}	Hilliker[b]	87D2-4; 87E1-2	–	–	–	–	–	–	+	+	+
Df(3R)ry^{1402}	Hilliker[b]	87D2-4; 87D14-E2	–	–	–	–	–	+	+	+	+
Df(3R)ry^{1607}	Hilliker[b]	87D3-4; 87E2-3	–	–	–	–	–	–	–	+	+
Df(3R)ry^{1608}	Hilliker[b]	87D4-6; 87E1-2	–	–	–	–	–	+	+	+	+
Df(3R)kar^{SZ8}	Gausz[a]	87C1-3; 87D14-15									
Df(3R)kar^{SZ33}	Gausz[a]	87C1-3; 87E4-5									
Df(3R)kar^{SZ37}	Gausz[a]	87C5-6; 87D14-E1									
Df(3R)kar^{1W}	Ish-Horowicz[c]	87A6-7; 87D13-14									
Df(3R)H5	Henikoff[d]	87A1-2; 87D5-7									
Df(3R)H10	Henikoff[d]	87A1-2; 87D6-7									
Df(3R)H13	Henikoff[d]	87B1-2; 87D14-E1									

The first nine deficiencies are derived from the present study. The next 14 have been mapped previously (SPIERER *et al.* 1983). The remaining seven have not been studied in detail, but are included for reference.

[a] GAUSZ *et al.* 1979.

[b] HILLIKER *et al.* 1980.

[c] ISH-HOROWICZ, HOLDEN and GEHRING 1977.

[d] HENIKOFF 1979.

[e] The full cytological description of this chromosome is *Df(3R)*87B3-5; 87D6-12, *In(3R)*87B-D; 99E1-F1.

The probes also hybridize to the balancer chromosome; this signal provides an internal control of hybridization. It also allows semiquantitative reading of the hybridization. When no signal is seen on the deficiency-carrying chromosome, we estimate that less than one-fifth of the DNA insert can hybridize to the chromosome. When the signal is positive, but strongly reduced compared to the balancer, we estimate that less than one-half of the DNA insert does hybridize. A similar signal on the balancer and on the deficiency-carrying chromosome indicates that more than one-half of the probe hybridizes to the deficiency-carrying chromosome. These rough estimates were confirmed in the instances where the breakpoint was mapped more precisely by genomic Southern blot hybridizations (BENDER, SPIERER and HOGNESS 1983; this work). Figure 1 shows examples of *in situ* hybridization to deficiencies, and the complete results are listed in Table 2. That the rearrangements are, indeed, deletions is shown by the failure of recombinants "within" the deletion to hybridize. Failure to hybridize was also found with recombinants further within the deletion in the process of nailing down the break (not shown). Short rearrangements at the breakpoint may, however, escape detection.

In some cases it was desirable to map the breaks more precisely, particularly in the proximal and distal regions of the walk where both genetic units and transcriptional activity seem to be concentrated. This was done by hybridization of recombinant DNA to Southern blots of genomic DNA from the deletion-bearing stocks. At the position of the break, DNA from the 87D,E region is fused to DNA at the other end of the deficiency, creating, in the majority of cases, a restriction fragment of different length to that found both in the wild type and in the balancer chromosome. Genomic DNA from the deficiency-bearing stocks and from an Oregon-R wild-type stock was digested with an appropriate restriction enzyme, separated by electrophoresis and transferred to nitrocellulose. Such "genomic blots" were then hybridized with recombinant phages from the region of the breakpoint predicted by *in situ* hybridization. To determine unambiguously which restriction fragment gave rise to the new fusion fragment, genomic blots were subsequently hybridized with individual restriction fragments (either subcloned or isolated from a gel). Oregon-R DNA should then exhibit a single band, whereas deficiency over balancer DNA exhibits two bands. Alternatively, the position of a fusion fragment can be determined if it hybridizes to two recombinant phages that share a small overlapping region.

Breakpoint positions were established by Southern hybridization for six of the new deficiences, as well as for three of the original deficiencies that had previously been characterized only by *in situ* hybridization. The DNA segments hybridizing to fusion fragments are listed in Table 3, and Figure 2 illustrates the results. In all cases the position determined by Southern hybridization was entirely consistent with the results of *in situ* experiments. Since Oregon-R is not the original stock on which the rearrangements were induced, the possibility remains that differences are due to DNA sequence polymorphism. The results of *in situ* hybridizations which attest that the breakpoints are, indeed, within a few kilobases renders this possibility unlikely.

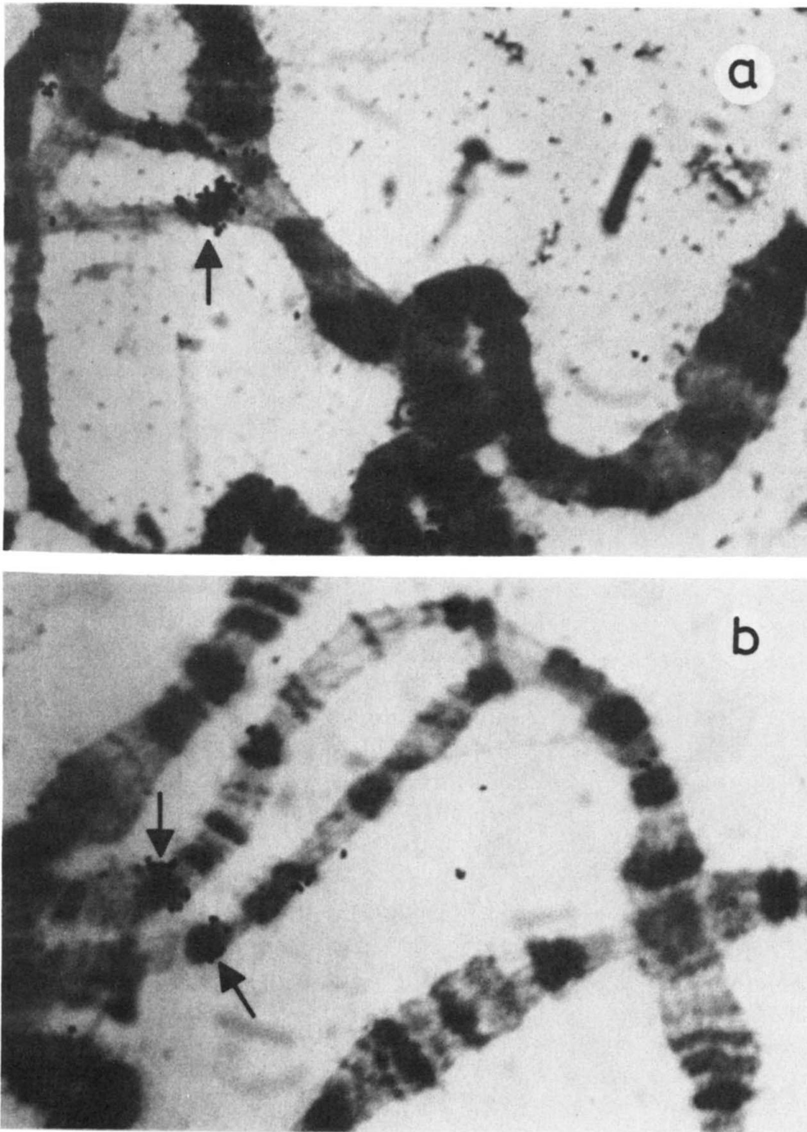

FIGURE 1.—Mapping deficiency breakpoints by *in situ* hybridization. Recombinant phage 2126 (−46; −29) was hybridized to salivary gland chromosomes from the stock *Df(3R)GE99/MKRS*. Autoradiography (panel a) shows grains on the balancer chromosome and not on the deficiency-bearing homologue. The same result was obtained with 2164 (−72; −54) (not shown). In contrast, the adjacent recombinant 2184 (−86; −70) labels both homologues of the same stock (panel b), thus placing the breakpoint within 2184.

Delineation of complementation groups: Mapping of the new deletion breakpoints permits us to define the position of complementation groups much more accurately than previously was possible. The maximum limits for each gene are defined by deficiencies that arrive from both sides and that do not

TABLE 2

Results of *in situ* hybridization to deficiencies

Deficiency breakpoint	Balancer	Closest positive[a] (walk coordinates)	Closest negative[b] (walk coordinates)
Df(3R)N40 distal	*MKRS* or *TM2*	2131 (+23, +41)	2121 (−1, +20)
Df(3R)N42 distal	*MKRS* or *TM2*	2160 (+63, +79)	2131 (+23, +41)
Df(3R)N63 distal	*TM2*	2160 (+63, +79)	2148 (+52, +68)
Df(3R)N69 distal	*MKRS*	2199 (−129, −111)	2821 (−141, −125)
Df(3R)N74 distal	*MKRS*	2821 (−141, −125)	2827 (−157, −139)
Df(3R)N78 distal	*TM2*	2148 (+52, +68)	1930 (+33, +51)
Df(3R)GE26 proximal	*MKRS* or *TM3*	2115 (−34, −17)[c]	2117 (−18, −1)
Df(3R)GE41 proximal	*MKRS*	2131 (+23, +41)	2160 (+63, +79)
Df(3R)GE99 proximal	*MKRS*		

[a] Recombinant phage closest to the breakpoint giving positive hybridization to the deficiency chromosome. (Phages are described in BENDER *et al.* 1983).

[b] Recombinant phage closest to the breakpoint failing to hybridize to the deficiency chromosome.

[c] Signal reduced relative to balancer.

TABLE 3

Results of Southern hybridization to genomic DNA from deficiencies

Deficiency breakpoint	Balancer	Control	Fragment disrupted[a] (walk coordinates)	Probe[b] (walk coordinates)
Df(3R)N40 distal	*MKRS*	Ore-R	*Hin*dIII-*Eco*RI (+3, +8.5)	*Hin*dIII-*Eco*RI (+3, +8.5)
Df(3R)N42 distal	*MKRS*	Ore-R	*Eco*RI-*Eco*RI (+60.5, +63.5)	*Eco*RI-*Eco*RI (+60.5, +63.5)
Df(3R)N63 distal	*TM2*	Ore-R	*Eco*RI-*Eco*RI (+73, +84)	2160/2192 (+72.5, +79)
Df(3R)N74 distal	*MKRS*	N42/MKRS	*Sal*I-*Sal*I (−143, −134.5)	*Eco*RI-*Sal*I (−138, −134.5)
Df(3R)N78 distal	*TM2*	Ore-R	*Eco*RI-*Eco*RI (+54, +55.5)	*Eco*RI-*Eco*RI (+54, +55.5)
Df(3R)GE26 proximal[c]			*Eco*RI − *Bam*HI (−30, −23)	2115 (−34, −17)
Df(3R)GE41 proximal	*MKRS*	Ore-R	*Eco*RI-*Eco*RI (+56.5, +58.8)	*Eco*RI-*Eco*RI (+56.5, +58.8)
Df(3R)ry[1301] distal	*MKRS*	ry^{+13}	*Bam*HI-*Bam*HI (+15, +21.5)	*Bam*HI-*Bam*HI (+15, +21.5)
Df(3R)ry[1607] distal	*MKRS*	Ore-R	*Bam*HI-*Bam*HI (+26, +32)	*Bam*HI-*Bam*HI (+26, +32)
Df(3R)l26d distal	*MKRS*	Ore-R	*Hin*dIII-*Hin*dIII (+79.5, +90.5)	2192/2189 (+84.5, +88.5)

[a] Restriction fragment in control and balancer DNA that in the deficiency contains a breakpoint, giving rise to an abnormal junction fragment (see Figure 2).

[b] Restriction fragment used to demonstrate the origin of a junction fragment.

[c] Deduced from transheterozygote flies bearing overlapping deficiencies (Figure 4).

disrupt gene function. Thus, the essential sequences for *S12, rosy* (see also DISCUSSION), *S8, G7* and *m32* are all mapped to within about 10–30 kb of DNA sequence, as shown in Figure 3. *Ace* is within 40 kb, *pic* is within 50 kb and

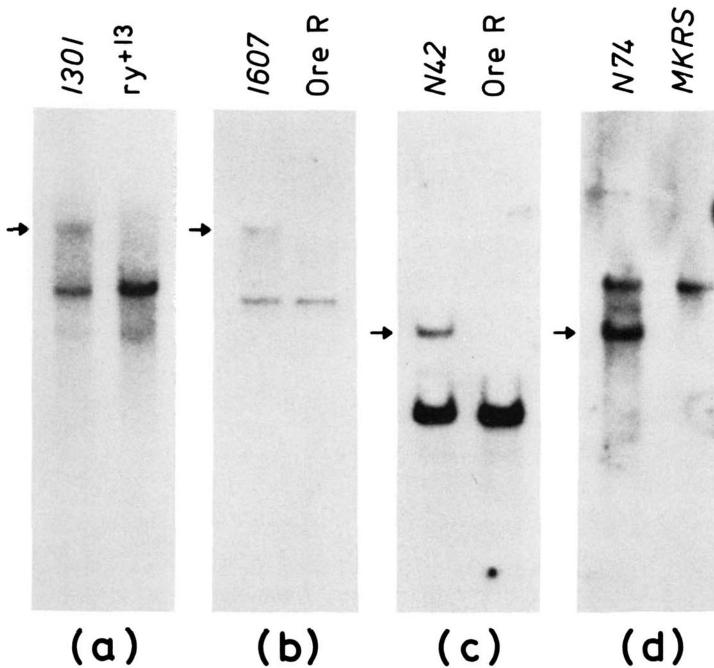

FIGURE 2.—Mapping deficiency breakpoints by Southern hybridization. Genomic DNA from flies of the genotypes described was digested with restriction enzymes, and the resultant genomic blot was hybridized with an appropriate restriction fragment, as described in Table 3. The figure illustrates the wild type and junction fragments (arrowed) which demonstrate the position of four different deficiency breaks. a, *Df(3R)ry*^{1301}*/MKRS* DNA and *ry*$^{+13}$ DNA digested with *Bam*HI and probed with a 6.5-kb *Bam*HI fragment (+15, +21.5). b, *Df(3R)ry*^{1607}*/MKRS* DNA and Oregon-R DNA digested with *Bam*HI and probed with a 5.8-kb *Bam*HI fragment (+26, +32). c, *Df(3R)N42/MKRS* DNA and Oregon-R DNA digested with *Eco*RI and probed with a 3-kb *Eco*RI fragment (+60.5, +63.5). d, *Df(3R)N74/MKRS* DNA and *Df(3R)N42/MKRS* DNA digested with *Sal*I + *Eco*RI and probed with a 3.5-kb *Eco*RI-*Sal*I fragment (−138, −134.5). (*N42/MKRS* DNA was used as a control because of restriction map heterogeneities in this region).

C9a and *B16-1* are within 95 and 120 kb, respectively (Figure 3). Deficiencies that do disrupt function give a further indication of the location of the gene, determining that some essential part of the gene lies within the deleted sequence. By this criterion we can localize at least some part of all genes, except *B16-1* and *C9a*, to about 10–20 kb of DNA; *C9a* lies within 40 kb (Figures 3 and 4). For *B16-1*, deficiencies coming from either side define essential sequences 50-kb apart; therefore, the *B16-1* gene must have a minimum size of about 50 kb (Figures 3 and 4).

Generation of overlapping deficiencies: The large set of deficiencies that we have now mapped raises the possibility of creating small homozygous deletions by constructing transheterozygotes of deficiencies entering the walk from opposite ends and having a small overlap. We have found that it is possible to generate flies with the overlapping combination *Df(3R)ry*^{1301}*/Df(3R)GE26* (which forms a homozygous deficiency for the complementation group *C9a*). The frequency of transheterozygotes of the two deficiencies is low (178 of 6095 total progeny), but nevertheless is comparable to the frequency of

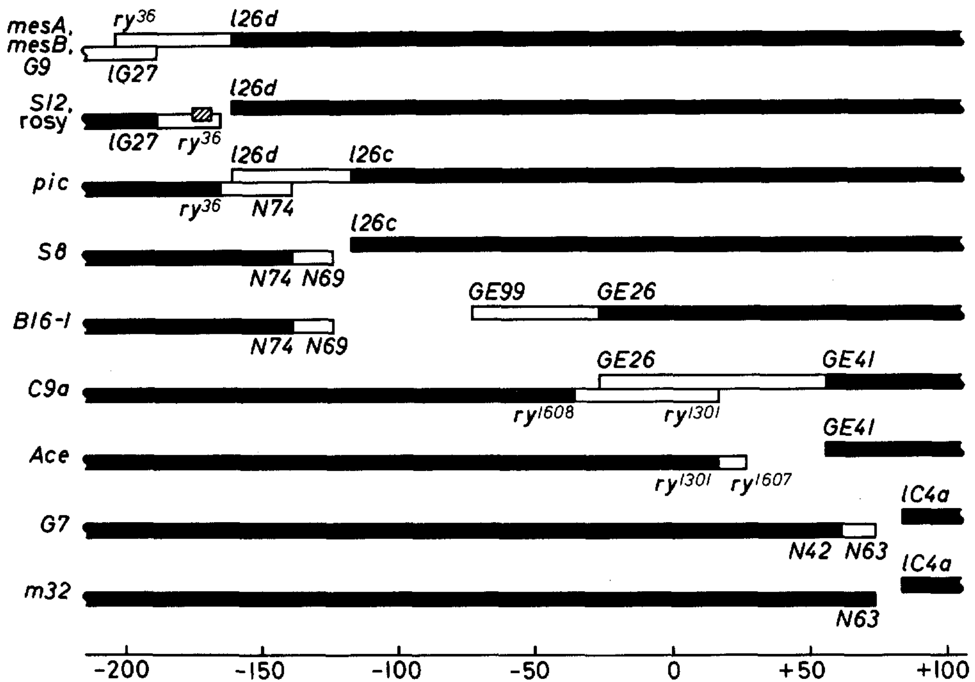

FIGURE 3.—Defining the limits of known complementation groups. Filled bars represent DNA not essential to each complementation group, as defined by the deficiencies indicated. Open bars represent DNA shown to contain sequences essential to each complementation group by the deficiencies indicated. The hatched box represents a DNA fragment that can rescue *rosy* and *S12* mutants (see DISCUSSION).

transheterozygote of a *C9a* point mutant *B2-6* over deficiency *Df(3R)GE26* (224 out of 5846). To establish that transheterozygous flies do, indeed, carry a deletion of the overlapping region, the DNA was analyzed by Southern hybridization. Genomic DNA was isolated from flies of the genotype *Df(3R)ry*^{1301}*/Df(3R)GE26*, and as a control from flies bearing *Df(3R)GE26/ MKRS*. Genomic blots of these DNAs were hybridized with recombinant phages covering the proposed deletion, as shown in Figure 4. As expected, the transheterozygote deficiency DNA contains two restriction fragments of anomalous length that correspond to the deficiency junction fragments, and it lacks a contiguous series of restriction fragments that are present in control DNA. Hybridization of the same filters to recombinant phages from elsewhere in the walk gave the normal pattern of restriction fragments in transheterozygote deficiency and control DNA (data not shown). The results confirm that flies of the genotype *Df(3R)ry*^{1301}*/Df(3R)GE26* do contain a homozygous deletion of DNA, which extends for 45 kb (or 40 kb excluding a transposable element) from walk coordinates -28 ± 2 to $+18 \pm 3$.

DISCUSSION

We have described the generation and mapping of nine new deficiencies with breakpoints in the *rosy-Ace* chromosomal walk. The breakpoints are dis-

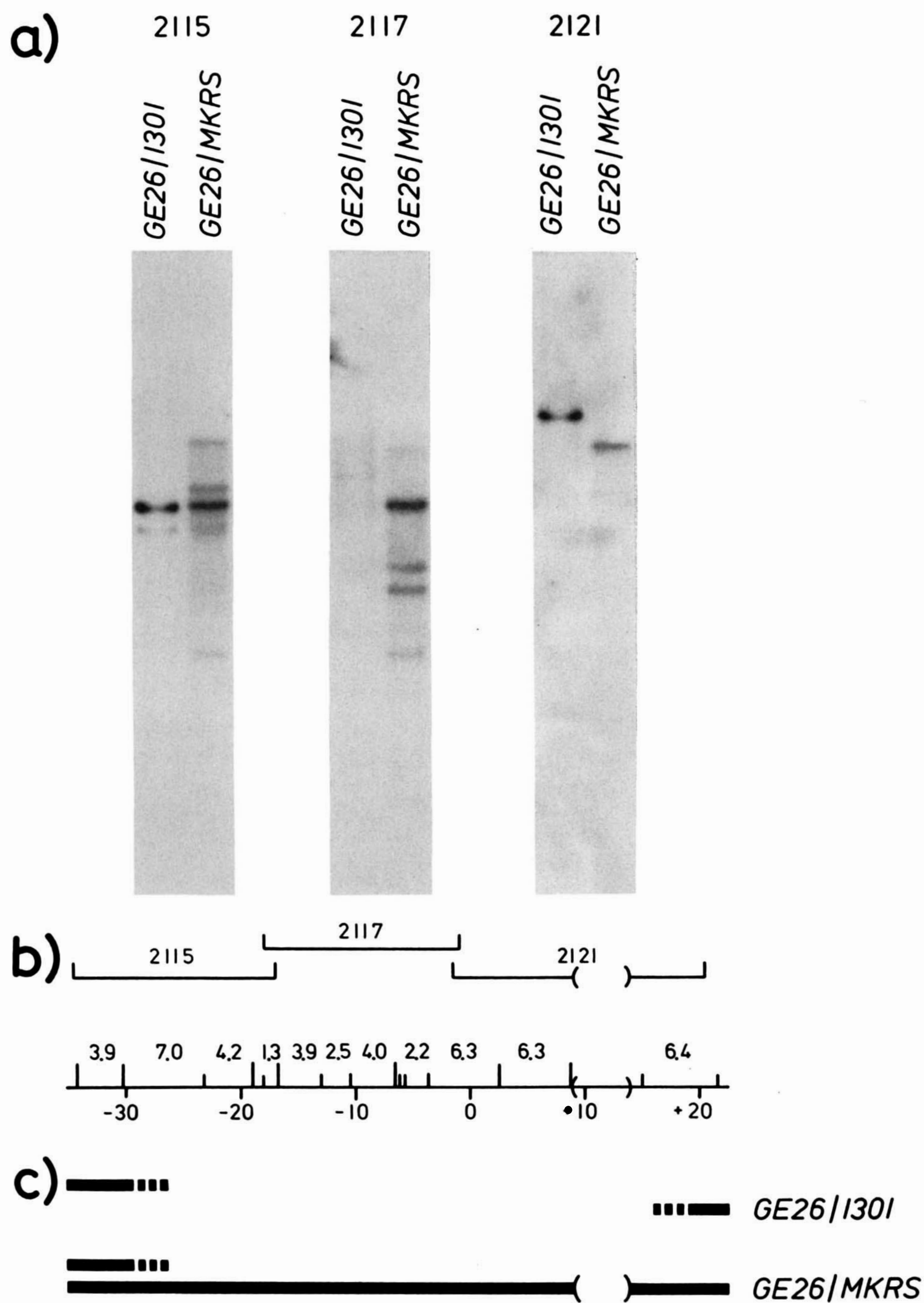

FIGURE 4.—Analysis of DNA from transheterozygous flies. a, Genomic blots of *Eco*RI + *Bam*HI digested DNA from flies of the genotypes *Df(3R)GE26/Df(3R)ry*^{1301} and *Df(3R)GE26/MKRS* hybridized with the recombinant phages 2115, 2117 and 2121. b, The position of the probes is illustrated relative to the DNA map of the region. *Eco*RI sites (tall marks) and *Bam*HI sites (short marks) are shown, together with the length of resultant restriction fragments. c, Structure of the two chromosomes from each genotype as deduced from the Southern blot (bars represent the extent of remaining DNA).

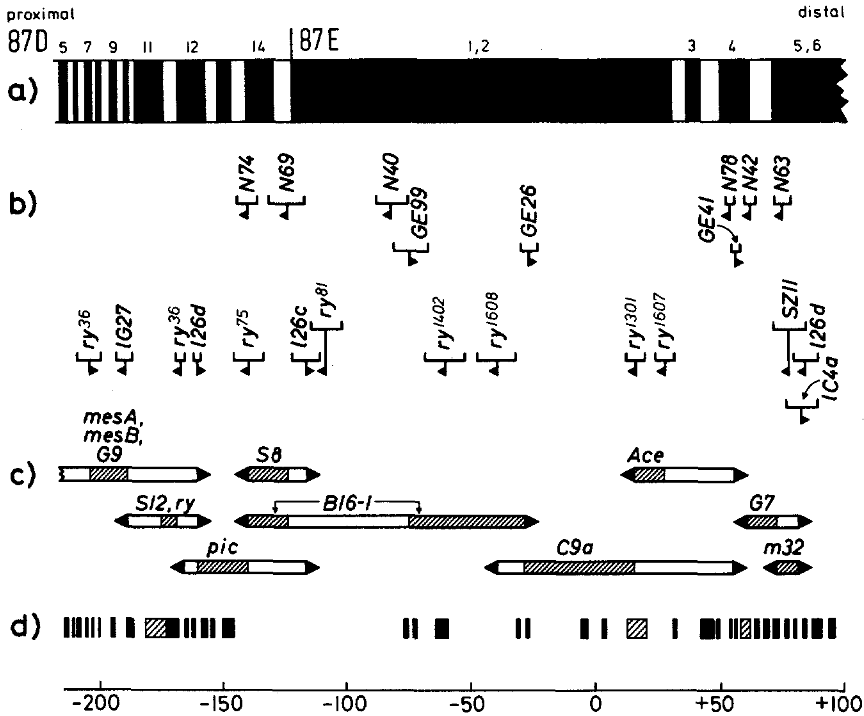

FIGURE 5.—Summary of genetic, cytological and molecular information on the region. a, A representation of the polytene chromosome as it corresponds to the DNA map (SPIERER *et al.* 1983). b, Position of all deficiency breakpoints mapped in this and previous studies. c, The maximum extent of each complementation group (bounded by triangles), with the position of sequences known to be essential (hatched); see also Figure 3. d, RNA transcripts mapped in various tissues and stages of development (BOSSY, HALL and SPIERER 1984; HALL, MASON and SPIERER 1983). Hatched boxes indicate that more than one transcript must be derived from a single DNA sequence. Coordinates in kilobases of DNA are shown below.

tributed broadly across the region, as are the breakpoints of deficiencies that had been mapped previously.

The new deficiencies serve to delineate the position of complementation groups in the region with a considerably improved resolution (see RESULTS). Having deletions entering the chromosomal walk from both ends allows us to map genes on the basis of positive results (how much DNA sequence can one delete without loss of gene function) rather than on negative results (which is the first break that abolishes function). This ensures that possible deleterious position effect associated with the fusion point will not be confused with the actual location of a gene. Figure 5 summarizes our accumulated data on the mapping of genes, bands and transcripts in the *rosy-Ace* walk. Such a relatively detailed map is essential to the long-term goal of characterizing particular genes within the walk. For instance, we have an interest in the localization of *Ace*, which has been proposed as the structural gene for acetylcholinesterase (HALL and KANKEL 1976). H. SOREQ and co-workers have recently collaborated with us to locate a sequence at +60 on the DNA map that hybrid-selects an

RNA capable of inducing acetylcholinesterase activity when injected into Xenopus oocytes (SOREQ *et al.* 1985). This sequence is now shown to lie outside the limits of the *Ace* gene, implying that more than one gene may be involved in the expression of active acetylcholinesterase in the fly.

It is also of considerable interest that ANDERSON and NÜSSLEIN-VOLHARD (1984) have mapped the position of *snake*, a maternal-effect gene involved in the establishment of dorsal-ventral pattern in the early embryo, within the *rosy-Ace* walk. The gene is disrupted by $Df(3R)ry^{36}$, but not by $Df(3R)kar^{1G27}$ (ANDERSON and NÜSSLEIN-VOLHARD 1984), placing it within the same 22-kb DNA segment as *S12* and *rosy*. Indeed, *l(3)S12* and *rosy* lie entirely within a 8.1-kb restriction fragment that rescues mutants of both genes following *P*-factor mediated transformation (RUBIN and SPRADLING, 1982 and A. CHOVNICK, personal communication), but it is not known whether *snake* maps to the left or right of these genes.

The delineation of genes within the walk has led also to the observation that *B16-1* extends over 50 kb or more of DNA. Such large genes appear to be rather rare in Drosophila, with notable exceptions being the genes of the *bithorax* complex, the *Antennapedia* complex and the *Notch* locus, which all have very large intervening sequences (BENDER *et al.* 1983; GARBER, KUROIWA and GEHRING 1983; SCOTT *et al.* 1983; KIDD, LOCKETT and YOUNG 1983; ARTAVANIS-TSAKONAS, MUSKAVITCH and YEDVOBNICK 1983). *B16-1* lies in the proximal half of the large band (or doublet) E1,2, probably spanning the proximal boundary of that band. This is in contrast to our earlier belief that there was no genetic activity over a large part of the left half of E1,2 (SPIERER *et al.* 1983; HALL, MASON and SPIERER 1983).

The right part of E1,2 contains *C9a* and, possibly, *Ace*. However, we have established that about 45 kb of DNA from the *C9a* segment can be deleted from both chromosomes and still yield a few fertile flies (*C9a* is only partially lethal). Thus, although between three and five transcripts have been mapped to the overlap, there appear to be no essential DNA sequences other than those encoding *C9a*. Our previous work had shown that there are many more transcripts than essential complementation groups in the region. As we have discussed previously (HALL, MASON and SPIERER 1983; BOSSY, HALL and SPIERER 1984), there are a variety of possible explanations for this apparent excess of transcripts: they could be products of genes that are not essential because they are repeated elsewhere in the genome, their functions are redundant or their functions are simply not required under normal culture conditions. There also may be an overestimate of transcript numbers due to multiple mRNAs being derived from differential processing of a primary transcript, although in regions where we have looked in detail, this is compensated numerically by rare transcripts not detected with the large probes used (L. M. C. HALL, unpublished data). Further, there is the possibility that some essential genes were missed by saturation mapping. The present evidence indicates that, in the limited case of the 45-kb homozygous deletion, the extra transcripts do not correspond to previously unrecognized essential genes. To ascribe a function, if any, to the many apparently active yet nonessential transcription units

remains a goal for the future, although the discovery that *snake* (ANDERSON and NÜSSLEIN-VOLHARD 1984) and a heat shock cognate gene (CRAIG *et al.* 1982; P. MASON, personal communication) map within the cloned region marks a step in that direction.

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