MOLECULAR MAPPING OF A GENE CLUSTER FLANKING THE DROSOPHILA DOPA DECARBOXYLASE GENE

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ABSTRACT

Nine lethal complementation groups flanking the *Drosophila* Dopa decarboxylase (*Ddc*) gene, have been localized within 100 kb of cloned chromosomal DNA. Six of these complementation groups are within 23 kb of DNA, and all ten complementation groups, including *Ddc*, lie within 78–82 kb of DNA. The potential significance of this unusually high gene density is discussed.

A random distribution of the estimated 5000–10,000 *Drosophila* genes (MULLER and PROKOFYEVA 1935; O'BRIEN 1973; BISHOP 1974) would result in an average spacing of one gene every 15–30 kb. However, analysis of the DNA flanking many cloned genes has revealed clusters of transcription units with densities of up to one transcript every 2–4 kb (histones: LIFTON *et al.* 1978; chorion proteins: SPRADLING 1981; heat shock proteins: CORCES *et al.* 1980; VOELLMY *et al.* 1981; SIROTKIN and DAVIDSON 1982; glue proteins: MEYEROWITZ and HOGNESS 1982; and cuticle proteins: SNYDER *et al.* 1982; SNYDER and DAVIDSON 1983). The relationship between this organization and gene regulation is unclear. Since these regions contain repeated gene families with extensive DNA homology, their organization may be the result of gene duplication and unequal crossing over.

Fifteen lethal complementation groups and the nonvital gene *hk* are tightly linked to the *Drosophila* Dopa decarboxylase (*Ddc*) gene. In contrast to other tightly clustered genes, there is no evidence that this region contains genes sharing extensive DNA sequence homology. These 17 complementation groups map within 0.045 map units and are contained within at most 7–11 polytene chromosome bands (WRIGHT *et al.* 1981).

Ddc, the best studied gene in this region, encodes an enzyme involved in the pigmentation and sclerotization of the cuticle as well as neurotransmitter synthesis. The gene is developmentally regulated (KRAMINSKY *et al.* 1980; MARSH and WRIGHT 1980; HIRSH and DAVIDSON 1981), exhibits tissue-specific expression (LUNAN and MITCHELL 1969) and may be hormonally regulated (KRAMINSKY *et al.* 1980; MARSH and WRIGHT 1980). Little is known about the function of the lethal complementation groups surrounding *Ddc*. However, *Ddc*, the nonvital gene *hk* and five of the lethal genes exhibit mutant phenotypes that

affect the production, sclerotization and/or pigmentation of the cuticle (WRIGHT *et al.* 1981; T. R. F. WRIGHT, unpublished results). For example, phenol oxidase activity is reduced in mutant alleles of *l(2)37Bf*, whereas hypersensitivity to a substrate analog of dopa decarboxylase results from mutations in *l(2)amd* (SPARROW and WRIGHT 1974). Altered catecholamine pool sizes are found in mutant alleles of both of these genes, as well as *Ddc* (B. BLACK, W. McIVOR and T. R. F. WRIGHT, unpublished results). In addition, somatic crossing over indicates that cuticular patches homozygous for *l(2)37Ca* contain shortened bristles, and leaky alleles of *l(2)37Cc* and *l(2)37Cf* result in abnormal bristles and alterations in cuticular pigmentation. The remaining complementation groups have no visible phenotype, being defined only as lethal alleles.

In this communication we report the cloning of 100 kb of chromosomal DNA adjacent to *Ddc* and demonstrate that the cloned DNA encodes nine of the 15 lethal complementation groups. Mapping several genetically defined breakpoints has localized these nine complementation groups plus *Ddc* to within 82 kb of DNA. Six of the complementation groups lie within at most 23 kb. This represents an exceptionally high gene density for *Drosophila*.

MATERIALS AND METHODS

Drosophila stocks: All mutations and balancer chromosomes used are described in LINDSLEY and GRELL (1968) unless otherwise noted. The wild-type strain, Canton S, was obtained from the *Drosophila* Stock Center, California Institute of Technology, Pasadena. *w TE42 Sco/B1 TE42* is homozygous for the transposable element *TE42* described by ISING and RAMEL (1976). Parent stocks and all other *Drosophila* stocks were obtained from the Charlottesville, Virginia, stock collection. *Df(2L)VA18*, a γ -ray-induced deletion, was isolated on the same *pr* chromosome and using the same protocol as *Df(2L)VA17* (WRIGHT *et al.* 1981). The genetic and cytological backgrounds of the deficiency strains referred to in this paper are summarized in Table 1.

Isolation of DNA: A library of Canton S *Drosophila* DNA cloned into the λ vector Charon 4 was obtained from J. LAUER (MANIATIS *et al.* 1978). The protocol to select specific λ -*Drosophila* hybrids from the library is that used by BENTON and DAVIS as outlined by MANIATIS *et al.* (1978). Phage particles were isolated and purified from *E. coli* KH802-infected cells as described by DAVIS, BOTSTEIN and ROTH (1980). The DNA was released from the phage particles with the addition of 50 μ g/ml of proteinase K and SDS to a final concentration of 0.2% and incubation at 65° for 15 min followed by 37° for 90 min. After sequential extractions with phenol-chloroform, Sevag, sec-butanol and ether the DNA was precipitated with isopropanol and resuspended in TNE (100 mM Tris, pH 7.5, 10 mM NaCl, 1 mM EDTA).

TABLE 1

Drosophila strains

Deficiency	Breakpoints	Reference
<i>Df(2L)130</i>	37B9-C1,2; 37D1,2	WRIGHT, HODGETTS AND SHERALD (1976)
<i>Df(2L)hk18</i>	36E4-6; 37B12-C1,2 ^a	This paper
<i>Df(2L)VA17</i>	37B9-C1,2; 37F5-38A1	WRIGHT <i>et al.</i> (1981)
<i>Df(2L)VA18</i>	36D1-E1; 37C1,2-C5	This paper
<i>Df(2L)VA12</i>	37C1,2-C5; 38B1,2-C1,2	WRIGHT <i>et al.</i> (1981)
<i>Df(2L)VA13</i>	37C1,2-C5; 38C1,2-D1,2	WRIGHT <i>et al.</i> (1981)
<i>Df(2L)TE42-1</i>	37C1,2-5; 38F41,2-D1,2	WRIGHT <i>et al.</i> (1981)

^aThe endpoints of *Df(2L)hk18* have been previously reported as 36E4-6; 37B9-C1,2 (BRITT-NACHER AND GANETZKY 1983).

All genomic *Drosophila* DNA fragments were inserted into the plasmid pBR322 and maintained in the *E. coli* strain C600. DNA was extracted as described by GUERRY, LEBLANC and FALKOW (1973). The plasmid DNA was purified by equilibrium centrifugation in cesium chloride-ethidium bromide gradients as outlined by MANIATIS, FRITSCH and SAMBROOK (1982).

Drosophila DNA was extracted from whole flies using a 2-ml Wheaton glass homogenizer. The procedure is a modification of that used by DAVIS as described by BENDER, SPIERER and HOGNESS (1983). To ensure that the DNA could be digested by restriction endonucleases the DNA was precipitated with spermine tetrahydrochloride (W. R. McCURE, unpublished results) followed by ethanol precipitation and final resuspension in TNE.

Gel electrophoresis and DNA transfer: *Drosophila* DNA was electrophoresed on vertical 0.7% agarose gels. Fragments were transferred to nitrocellulose filters as described by SOUTHERN (1975) in 20× SSPE (3 M NaCl, 0.2 M NaH₂PO₄, 0.01 M Na₂EDTA). DNA from approximately ten flies was loaded in each lane.

Hybridization: DNA restriction fragments to be used as hybridization probes were electroeluted from 0.7% agarose gels by one of two methods. In the first, the desired band was cut out of the gel, the gel slice placed in the apparatus described by ALLINGTON *et al.* (1978) and the DNA electroeluted onto dialysis membrane. Modifications in the procedure include using 0.5× E in the inner chambers and 10× E in the outer chambers, as well as the addition of 1 µg of tRNA to all membranes to serve as carrier. In the second method, the DNA was restricted and electrophoresed on a horizontal 0.7% agarose gel in the presence of 1 µg/ml of EtBr. A well was cut just beyond the fragment to be isolated, the bottom of the well and the far wall were covered with dialysis membrane, the well was filled with 1× E and the DNA was electrophoresed into the well onto the membrane. In both procedures the resultant DNA preparation was phenol-chloroform extracted, ethanol precipitated and resuspended in TNE.

DNA to be used as hybridization probes was labeled with α³²P by the nick translation procedure described by MANIATIS, JEFFREY and KLEID (1975). Labeled probe was recovered in the excluded fraction of a BioGel P-60 column. The peak fractions were pooled, phenol-chloroform extracted, ethanol precipitated and resuspended in TNE.

Nick-translated DNA, 10⁵–10⁶ dpm, was hybridized to filter-bound phage DNA in 50% formamide, 1 M NaCl, 50 mM Tris, pH 8.3, 1 mM EDTA, 5% Denhardt's solution, 50 µg/ml of denatured carrier DNA and 8% dextran sulfate. All filters were prehybridized in the same solution without dextran sulfate. All hybridizations were performed at 42°. Filters were washed at 68° for 15 min in 0.1 M NaCl, 50 mM Tris, pH 8.3, 1 mM EDTA, 0.1% SDS, 0.1% NaPPi, 1× Denhardt's solution and up to 2 hr in the same solution without Denhardt's.

In situ hybridization: Salivary glands were dissected and slides prepared as described by BONNER and PARDUE (1976) except that the slides were denatured in 0.1 N HCl. ³HcRNA was prepared according to the procedure of WENSINK *et al.* (1974). cRNA was hybridized to the chromosome squashes in 50% formamide, 0.1 M EDTA, 0.02 M Pipes, pH 7.4, and 1 M NaCl. Hybridization mix, 10 µl, was placed on each slide and covered with a round acid washed, siliconized coverslip (15 mm diameter). Hybridization was performed at 37° in air-tight boxes saturated with 1 M NaCl, 50% formamide. Slides were washed in 1 M NaCl, 50% formamide for a total of 45 min, 2× SSPE for 30 min and air dried. Autoradiographic emulsion (Kodak NTB-2) was used as described by PARDUE and GALL (1975).

RESULTS

Genetics and cytology of the region flanking Ddc: *In situ* hybridization places *Ddc* in or near polytene chromosome band 37C1,2, on the left arm of chromosome 2 (HIRSH and DAVIDSON 1981). The cytological locations of the complementation groups adjacent to *Ddc* and the relevant deficiency breakpoints are summarized in Figure 1. Screens for lethals deleted by *Df(2L)130* have identified 17 loci: *Ddc* and 15 lethal complementation groups in addition to the nonvital gene *hk* (WRIGHT *et al.* 1981, *l(2)37Bf*, *Bg* and *Cg* have not been previously reported). Complementation analysis with *Df(2L)VA17* and *Df(2L)VA18* places

FIGURE 1.—Location of deficiency breakpoints and lethal complementation groups within the 37B9-D1,2 chromosomal region. Salivary gland polytene chromosome bands are drawn schematically as described by SAURA and SORSA (1979). The thickness of the vertical line corresponds to the relative band thickness, with faint bands represented by dashed lines. The extent of a deletion is indicated by a solid horizontal line, and the uncertainty in the cytological location of the breakpoint is indicated by a horizontal dashed line. The position of the 15 lethal complementation groups, *hk* and *Ddc* is shown with respect to the deletion endpoints. Although the reported cytological location of the distal breakpoint of *Df(2L)130* (37B9-C1,2) is the same as the proximal breakpoint of *Df(2L)hk18*, *Df(2L)hk18* deletes only six of the nine loci distal to *Ddc*, whereas *Df(2L)130* deletes all nine. To show this difference between the cytological and genetic maps these breakpoints are shifted within their respective interband region. *l(2)Bf*, *Bg* and *Cg* have not been previously reported.

these lethal complementation groups on the centromere proximal or centromere distal side of *Ddc*. *Df(2L)TE42-1* and *Df(2L)hk18* further subdivide the *Ddc* proximal and *Ddc* distal lethal complementation groups, respectively. *Df(2L)VA18* is a new γ -ray-induced deficiency (36D1-E1; C1,2-C5), deleting *Ddc*, all distal lethal complementation groups shown and *hk*.

The approximate cytological location of these breakpoints (WRIGHT *et al.* 1981; BRITTNACHER and GANETZKY 1983) places the six loci proximal to *Ddc* within a region containing one to three chromosomal bands: 37C1,2 (37C1,2 is treated as a single band; SAURA and SORSA 1979), 37C3 and 37C4. The three loci distal to *Ddc* are within a region containing one to five chromosomal bands: 37B10, 37B11, 37B12, 37B13 and 37C1,2. More than 150 lethal mutations have been isolated in these nine complementation groups in addition to 50 mutations in *Ddc* (WRIGHT *et al.* 1981; T. R. F. WRIGHT, unpublished results). Of the 109 mutations proximal to *Ddc*, 108 mutations have been

assigned unequivocally to the six lethal complementation groups: *i.e.*, $l(2)37Ca$ with 38 alleles, $l(2)37Cb$ with 31, $l(2)37Cc$ with 21, $l(2)37Cd$ with ten, $l(2)37Ce$ with six and $l(2)37Cg$ with two alleles. Of the possible 5886, 2299 *inter se* crosses between the 108 mutations have been completed with no evidence of noncomplementation between the six complementation groups. Only one mutation recovered in this region is noncomplementing with some alleles from two of these six complementation groups. Investigation of this semilethal mutation is incomplete (T. R. F. WRIGHT, unpublished results). In spite of this one mutation, the genetic data along with preliminary data on mutant phenotypes suggest that there are six distinct tightly linked genes with separate functions in this region of the genome.

Cloning the DNA flanking Ddc: All *Drosophila* DNA clones were selected from a library containing λ -*Drosophila* hybrid phage. The phage library (MANIATIS *et al.* 1978) contains randomly sheared wild-type Canton S DNA inserted into the λ vector, Charon 4. Single-copy restriction fragments from existing cloned *Drosophila* DNAs (HIRSH and DAVIDSON 1981) were used as probes to select phage containing overlapping *Drosophila* DNA inserts. Restriction endonuclease sites were mapped on the newly isolated *Drosophila* DNA, and the process was repeated. The 100 kb of chromosomal DNA resulting from this sequential walk is diagrammed in Figure 2.

The orientation of the cloned DNA with respect to the centromere is known (GILBERT and HIRSH 1981). The data shown in Figure 3 confirms this orientation while further defining the cytological location of various segments of cloned DNA. 3H cRNA synthesized from the λ clones was hybridized *in situ* to polytene chromosome squashes from Canton S larval salivary glands. $\lambda 1$, containing DNA encoding *Ddc* (HIRSH and DAVIDSON 1981), hybridizes near or within polytene chromosome band 37C1,2. $\lambda 13$, containing DNA 13–26 kb centromere proximal from *Ddc* and $\lambda 16$ containing DNA 25–40 kb proximal from *Ddc*, both hybridize between 37C1,2 and 37D1,2. $\lambda 20$, containing DNA 45–60 kb distal to *Ddc*, hybridizes slightly distal to 37C1,2.

FIGURE 2.—Location of *Drosophila* DNA contained within λ -Canton S hybrid clones. Coordinate zero is arbitrarily assigned to the centromere proximal end of $\lambda 1$. Each coordinate unit corresponds to 1 kb of *Drosophila* DNA. All centromere distal DNA is assigned a negative number and centromere proximal DNA a positive number.

FIGURE 3.—*In situ* hybridization of selected probes to giant (*gt*) salivary gland chromosome squashes. The region between polytene chromosome bands 37A-D is shown. ^3H cRNA was made from λ -Canton S hybrid phage DNA as described by WENSINK *et al.* (1974) and used as probes. The position of the *Drosophila* DNA inserts on the chromosomal DNA map is shown in Figure 2.

Strategy for mapping breakpoints: Figure 1 illustrates the position of the lethal complementation groups relative to existing deficiency breakpoints. We have mapped these breakpoints on the cloned DNA, thus identifying the DNA sequences associated with the respective lethal phenotypes.

Restriction endonuclease digests of genomic DNA from all relevant *Drosophila* stocks were electrophoresed on agarose gels, transferred to nitrocellulose, and hybridized with ^{32}P -labeled DNA probes. Since the deletions studied are homozygous lethal, the genomic DNA was isolated from heterozygotes. The disappearance of restriction fragments within the deletion is not observed due to the presence of the wild-type homolog. Thus, deletion endpoints were localized by using DNA probes that recognize new restriction fragments representing the fusion of DNA flanking the deletion. Initially, the probes were constructed from the λ -*Drosophila* hybrid clones shown in Figure 3. To achieve a finer localization of the breakpoints, restriction fragments from relevant λ clones were subcloned into plasmid vectors and then used as probes.

Breakpoints proximal to Ddc: Six lethal complementation groups map within a chromosomal region defined on one end by *Ddc* and on the other end by the insertion of the transposable element *TE42* (ISING and RAMEL 1976) and the distal breakpoints of *Df(2L)VA12* and *Df(2L)VA13*. Figure 4 diagrams the location of these breakpoints on the cloned DNA.

TE42 is a large transposable element containing the cytologically visible polytene chromosome bands 3C2,3 and 3C5,6. Salivary gland chromosome squashes (GILBERT and HIRSH 1981) and genomic DNA blots probed with the λ -*Drosophila* hybrid clones have localized *TE42* to the *Drosophila* DNA shared by $\lambda 13$ and $\lambda 16$ (Figure 2; coordinates 25.5–28.1). As shown in Figure 4, subclone 1053, which contains this DNA, hybridizes to the *Hind*III fragment into which the insertion occurred. The 2.6-kb *Hind*III fragment in the DNA from the control strain, Oregon-R, is not present in the homozygous insertion strain. Instead, the 1053 probe hybridizes to two new fragments, each of greater than 12 kb. These two fragments presumably represent the DNA flanking the insertion joined to the DNA from the ends of *TE42*.

Standard genetic techniques have placed the distal endpoints of the deletions

FIGURE 4.—Localization of breakpoints proximal to *Ddc* on the chromosomal DNA. A, Genomic DNA blots of *Drosophila* DNA were probed with ^{32}P -labeled DNA fragments. The resultant pattern for each deficiency strain is compared with its parental (P) strain. (1) A *Hind*III digest of the strain homozygous for the insertion of the transposable element *TE42*, *w TE42 Sco/B1 TE42*, and its parent strain, Oregon R (P), was probed with subclone 1053. (2) An *Eco*RI digest of the deficiency heterozygote *Df(2L)VA12/CyO* and its *cn bw* parent strain (P) was probed with 1051 and (3) 1053. (4) A *Hind* III digest of the deficiency heterozygotes *Df(2L)VA13/CyO*, *Df(2L)130/CyO* and the *cn bw* parent strain (P) was probed with subclone 1056. Since *Df(2L)130* contains a deletion removing all of the cloned DNA discussed it is shown as —/CyO. B, The location of the DNA probes and breakpoints with respect to the balancer, *CyO*, and parent chromosomal DNAs is diagrammed. The restriction endonuclease map of *CyO* for the enzymes *Eco*RI, ($\downarrow$), and *Hind*III, ($\uparrow$), is shown. Only those additional restriction sites not in *CyO* are indicated for the parent strains (P). The solid horizontal line indicates deleted DNA, and the dashed line indicates uncertainty in the position of the breakpoints.

Df(2L)VA12 and *Df(2L)VA13* near *TE42*. The position of these breakpoints on the DNA agrees with their genetic localization. The genomic blots in Figure 4 show a 6.5-kb *Eco*RI fragment in *Df(2L)VA12/CyO* when probed with the subclones 1051 or 1053, which is not present in the parent strain. Thus, the

deletion endpoint is between coordinates 25.5 and 28.1. The distal endpoint of *Df(2L)VA13* is between coordinates 30.7 and 35.4. Subclone 1056 detects a 4.7-kb *HindIII* fragment not present in the parent strain. For this fragment to be 4.7 kb, the deletion endpoint can be no further than 4.7 kb distal to the *HindIII* site at coordinate 30.7 or coordinate 35.4. The balancer, *CyO*, contains a restriction site polymorphism yielding a 13-kb *HindIII* fragment in the deletion heterozygote instead of the 7-kb *HindIII* fragment seen in the parent strain, *cn bw*. The positions of these endpoints have been verified by digestions with other restriction enzymes as well as hybridization with adjacent and overlapping subclones (not shown).

Breakpoints within Ddc: Two *Ddc*⁻ γ -ray-induced deletions have genetically defined endpoints which map between *Ddc* and the adjacent complementation groups. Genetic analysis indicates that *Df(2L)VA17* deletes all or part of *Ddc* and all of the centromere proximal lethal complementation groups shown in Figure 1. It does not remove *l(2)amd*, a recessive lethal, mapping 0.002 map units distal to *Ddc* (WRIGHT *et al.* 1981). *Df(2L)VA18* deletes *Ddc* and all recessive lethals distal to *Ddc* as shown. It does not delete *l(2)37Ca* which maps 0.008 map units proximal to *Ddc* (WRIGHT *et al.* 1981).

The restriction map of the chromosomal DNA adjacent to *Ddc*, the genomic blots and probes used to localize these deficiency endpoints are presented in Figure 5. *Df(2L)VA17* removes the DNA sequences containing the first 5' exon of *Ddc* (SCHOLNICK, MORGAN and HIRSH 1983, C. BEALL, B. A. MORGAN, G. MORSE and J. HIRSH, unpublished results). The 650-base pair (bp) insert from the subclone 1013 hybridizes to an 8.2-kb *EcoRI* and 8.2-kb *BamHI* fragment in DNA isolated from *Df(2L)VA17/CyO* but not from the parent strain. This is in addition to the expected 5.6-kb *EcoRI* and 5.5-kb *BamHI* fragment present in all nondeleted homologs. Experiments using overlapping and/or adjacent probes, 1004, 1022, 1023, (not shown) confirm the placement of the breakpoint to within the 650 base pairs represented by subclone 1013.

The proximal endpoint of *Df(2L)VA18* is between coordinates 5.1 and 5.6, entirely removing *Ddc*. An *EcoRI* genomic digest of DNA from *Df(2L)VA18/CyO* and the parent strain, hybridized with the subclones 1023 and 1071, shows the expected fragments as well as a new 5.6-kb *EcoRI* fragment. Given the size of this new fragment, the endpoint can be no further then 5.6 kb to the left of the *EcoRI* site at coordinate 10.7. In addition, the polymorphic 0.8-kb *HindIII* fragment found in the parent but not the balancer, *CyO*, (coordinates 5.6–6.4) remains in *Df(2L)VA18/CyO* (not shown).

A series of γ -ray-induced *Ddc* lethal alleles were screened for restriction pattern alterations. One allele *Ddc*ⁿ²⁷, was found to contain a 2.3-kb deletion (Figure 5). Two subclones containing adjacent *BamHI* fragments, 1022 and 1023, both hybridize to a 4.5- to 5-kb *BamHI* fragment unique to *Ddc*ⁿ²⁷/*CyO*. Therefore, the entire deletion is within the DNA covered by these two subclones (coordinates 0.1–7.3), and each subclone must recognize a separate endpoint. Further analysis has revealed that the deletion results in a 9.6- to 10.5-kb *EcoRI* fragment and a 4.7- to 5-kb *HindIII* fragment (not shown). The DNA contained within subclones 1013 and 1038 is deleted, and the poly-

FIGURE 5.—Localization of breakpoints within *Ddc* on the chromosomal DNA. A, Genomic DNA blots of *Drosophila* DNA were probed with ^{32}P -labeled DNA fragments. (1) An *Eco*R1 and (2) a *Bam*H1 digest of the deficiency heterozygote *Df(2L)VA17/CyO* and its *pr* parent strain (P) was probed with the isolated insert from subclone 1013. (3) An *Eco*R1 digest of the deficiency heterozygote *Df(2L)VA18/CyO* and its *pr* parent strain (P) was probed with subclone 1023 and (4) 1071. (5) A *Bam*H1 digest of the heterozygous lethal allele *Ddcⁿ²⁷/CyO* and its *cn bw* parent strain (P) was probed with subclones 1023 and (6) 1022. B, The location of the DNA probes and breakpoints with respect to the balancer, *CyO*, and parental chromosomal DNAs is shown. The restriction endonuclease map of *CyO* is given [*Eco*R1, ($\downarrow$), *Bam*H1, ($\uparrow$) and *Hind*III, ($\diamond$)]. The solid horizontal line indicates deleted DNA, with the dashed line indicating the uncertainty in the position of the breakpoints.

morphic 0.8-kb *Hind*III fragment is not deleted. These data are consistent with the deletion removing DNA between coordinates 1.1 and 3.4. This DNA encodes the first two 5' exons of *Ddc* and about 500 bp of flanking sequences (C. BEALL, B. A. MORGAN, G. MORSE and J. HIRSH, unpublished results).

Breakpoints distal to Ddc: Three lethal complementation groups map between *Ddc* and the proximal endpoint of *Df(2L)hk18*. The data shown in Figure 6 place these three complementation groups between the 3' end of *Ddc*, at coordinate -2 and the proximal breakpoint of *Df(2L)hk18*, at coordinate -51.4 to -53. Due to the lack of deficiency breakpoints in this region, the relative placement of these three lethal complementation groups within these 49-51 kb cannot be determined. A sample of the genomic blots and the resultant restriction maps are presented in Figure 6. The 3.0-kb *Eco*R1 fragment of λ 20, subclone 1087, detects a 3.6-kb *Eco*R1 and a 3.9-kb *Hind*III fragment, both unique to the deletion heterozygote. Since subclone 1087 hybridizes to the deleted 3.9-kb *Hind*III fragment with an intensity equal to the parental 4.9-kb *Hind*III fragment of *Df(2L)hk18*, the deletion can remove no more than half of the parental fragment. *In situ* hybridization (not shown) confirms that these fragments are the result of the deletion breakpoint.

DISCUSSION

Distribution of the lethal complementation groups on the DNA: We have isolated 100 kb of chromosomal DNA flanking the *Drosophila Ddc* and mapped break-

FIGURE 6.—Localization of the breakpoint distal to *Ddc* on the chromosomal DNA. Left, (1) An *Eco*R1 restriction endonuclease digest and (2) a *Hind*III digest of *Drosophila* DNA from the deficiency heterozygote *Df(2L)hk18/CyO* and its parent strain (*P/CyO*) was probed with 32 P-labeled DNA from subclone 1087. Right, The location of subclone 1087 and the breakpoint of *Df(2L)hk18/CyO* with respect to the chromosomal DNA is shown. Both the deficiency heterozygote and the parent strain have the same restriction maps. The solid horizontal line indicates deleted DNA, and the dashed line indicates the uncertainty in the position of the breakpoints.

points localizing ten lethal complementation groups, including *Ddc*, within this DNA. Six of these complementation groups are within 23 kb and the entire ten complementation groups included in the cloned DNA map within a 78- to 82-kb segment of DNA.

As summarized in Figure 7, the centromere proximal half of the cloned DNA contains six lethal complementation groups between the endpoint of *Df(2L)VA18*, at coordinates 5.1–5.6, and the insertion of the transposable element, *TE42*, at coordinate 23.5–28.1, or a maximum of 23 kb. If one includes *Ddc*, the next most distal lethal complementation group, seven lethal complementation groups are within 31 kb. The distal half of the cloned DNA contains three lethal complementation groups between the 3' end of *Ddc*, at coordinate -2, and the proximal breakpoint of *Df(2L)hk18*, at coordinate -51.4 to -53, or 49–51 kb. The six lethal complementation groups deleted by *Df(2L)hk18* are distal to coordinate -53 and are more than 50 kb from *Ddc*.

Two assumptions must be made in order to conclude that the region flanking *Ddc* contains an unusually high gene density. First, we assume that the number of lethal complementation groups accurately represents the number of genes. Second, we assume that the *Drosophila* genome contains on average 15–30 kb of DNA per gene.

The number of lethal complementation groups may represent an underestimate of the gene number. Alleles in genes with a low mutation frequency or

FIGURE 7.—Location of the polytene chromosome bands, deficiency breakpoints and lethal complementation groups with respect to the isolated *Drosophila* DNA. DNA from coordinates -45 to -60 contains the proximal breakpoint of *Df(2L)hk18* and is located between 37B11,12 and 37C1,2 (see DISCUSSION). DNA from coordinates 25.5–28.1 contains the proximal breakpoint of *Df(2L)VA12* and the insertion of *TE42* and is located between 37C1,2 and 37C5. *Ddc* is located in or near polytene chromosome band 37C1,2.

in genetically duplicated regions would not have been recovered. In addition, the mutation screens were designed to detect only recessive lethals or female steriles and not mutations in nonessential loci. It is doubtful that intragenic complementation has resulted in an overestimation of gene number, since most of the complementation group contains many more alleles than expected for an event as rare as intragenic complementation (WRIGHT *et al.* 1981).

The second assumption, that there is an average of 15–30 kb of DNA per gene, is based on an estimated 5000–10,000 genes distributed among 1.6×10^8 bp of euchromatic DNA (RUDKIN 1961). These estimates of gene number result from the study of mutational frequencies (MUKAI 1964; MUKAI *et al.* 1972), the saturation of small regions with lethals (LIFSCHYTZ and FALK 1968, 1969; JUDD, SHEN and KAUFMAN 1972; HOCHMAN 1973; LEFEVRE 1973) and measurements of polyadenylated RNA complexity (LEVY, JOHNSON and MCCARTHY 1976; IZQUIERDO and BISHOP 1979). Although, the first two methods only consider genes with lethal alleles, all three methods agree that there are between 5000 and 10,000 genes.

Distribution of lethal complementation groups on polytene chromosomes: The 17 genes, including *Ddc* and *hk*, are located in a seven- to 11-band region delineated by the breakpoints of *Df(2L)130* (37B9-C1,2;37D1-2). The cytological distribution of these genes is such that some regions contain a higher density of essential genes than others (Figure 1). The region including 37B10-37B13 (defined by breakpoints of *Df(2L)130* and *Df(2L)hk18*) contains five lethal complementation groups and the nonvital gene *hk* in one to four bands. 37B10-37C1,2 (defined cytogenetically by breakpoints of *Df(2L)hk18* and *Df(2L)VA17*) contains three lethal complementation groups in a maximum of five bands. However, the combined molecular and cytogenetic analysis presented in this communication suggests that the proximal endpoint of *Df(2L)hk18* is not 37B9-C1,2 but 37B12-C1,2. As shown in Figure 4, $\lambda 20$, containing the proximal breakpoint, hybridizes *in situ* on or just distal to 37C1,2. The results of additional *in situ* hybridizations (not shown) suggest that $\lambda 20$ hybridizes proximal to 37B11,12. Thus, these three complementation groups may be within at most two bands, 37B13 and 37C1,2. The region including 37C1,2-37C4 (defined by breakpoints of *Df(2L)VA17* and *Df(2L)TE42-1*) contains seven genes in one to three bands. Finally, 37C5-37D1 (defined by *Df(2L)TE42-1* and *Df(2L)130*) contains one lethal complementation group in five to seven bands. These results add to the growing evidence (BELYAEVA *et al.* 1980; LEFEVRE 1981; ZHIMULEV *et al.* 1981) that there can be a large variation in the number of genes per band.

Significance of a high gene density: To date, all regions characterized by a high gene density contain the members of a multigene family. The five histone genes are within a 4.8- to 5-kb repeating unit (LIFTON *et al.* 1978), four heat shock proteins at 67B are within 11 kb (CORCES *et al.* 1980; VOELLMY *et al.* 1981; SIROTKIN and DAVIDSON 1982), two of the transcribed regions at 7C and 66D encode chorion proteins and are within 7 and 10 kb, respectively (SPRADLING 1981), and of the four transcription units within 7.9 kb at 44D at least three encode cuticle proteins (SNYDER *et al.* 1982). The regulatory sig-

nificance of this nonrandom distribution is unclear, since some of these regions also contain interspersed genes unrelated in either DNA sequence or pattern of developmental regulation (SPRADLING 1981; SIROTKIN and DAVIDSON 1982; SNYDER and DAVIDSON 1983).

The region flanking *Ddc* differs from other gene clusters in that under normal hybridization conditions there is no detectable intergenic sequence homology (Figures 4–6; D. GILBERT and J. HIRSH, unpublished results). Since these preliminary experiments indicate that these genes are not evolutionarily related, an explanation for their close proximity may come from an examination of their functions as well as patterns of expression.

The functions of most of the 17 genes in this region are unknown. However, a least seven of the genes (*hk*, *Ddc*, *l(2)amd*, *l(2)37Ca*, *l(2)37Cc* and *l(2)37Cf*) affect the production, sclerotization and/or pigmentation of the cuticle. *l(2)37Ca* and *l(2)37Cc* are within the cluster of six lethal complementation groups proximal to *Ddc*. *l(2)amd* is one of the three lethal complementation groups within 50 kb distal to *Ddc*, whereas *hk* and *l(2)37Bf* are greater than 50 kb distal to *Ddc*.

Two lines of evidence suggest that the entire 16-gene region need not be intact for the proper expression of each of its members. First, the 23 kb containing *l(2)37Ca*, *Cb*, *Cc*, *Cd*, *Ce* and *Cg* do not require the surrounding DNA for expression. Strains containing *Df(2L)VA18* (deleting distal DNA) or *Df(2L)VA12* (deleting proximal DNA) complement mutations in any of these six complementation groups. Similarly, the DNA proximal to *Ddc*, deleted by *Df(2L)VA17*, is not necessary for the expression of any of the complementation groups distal to *Ddc*. Second, *P* element-mediated reintegrations of a DNA fragment encoding *Ddc* into chromosomal regions other than 37C1,2 (SCHOLNICK, MORGAN and HIRSH 1983) indicate that *Ddc* can be correctly expressed when separated from this gene cluster.

Recently, we have identified a large number of transcription units adjacent to *Ddc* (D. GILBERT and J. HIRSH, unpublished results). Examination of their pattern of expression may reveal the biological rationale for this region's high gene density.

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