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Molecular Mapping of Genetic and Chromomeric Units in *Drosophila melanogaster*

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We have used a set of overlapping cloned segments defining a 315 kb ($\times 10^3$ base-pairs) region of *Drosophila melanogaster* chromosomal DNA to map the sequences associated with the polytene band–interbands (chromomeric units) and with the lethal complementation groups contained within this region. The molecular map positions of the 13 ± 1 chromomeric units from the 87D5–6 to 87E5, 6 region of the third chromosome were determined by *in situ* hybridization of selected segments to the polytene chromosomes. The length of the largest chromomeric unit within the 315 kb region is approximately 160 kb, while that for the smallest is less than 7 kb and may be as short as 3 kb. By mapping the breakpoints of deletions within the 315 kb region, we have located its 12 lethal complementation groups, which include the genes coding for acetylcholinesterase (*Ace*) and xanthine dehydrogenase (*rosy*). Comparison of the two molecular maps indicates a one-to-one topographical correlation between the genetic and chromomeric units.

1. Introduction

The chromosomal walk described in the accompanying paper (Bender *et al.*, 1983) has provided us with a set of overlapping cloned segments that cover 315 kb‡ of DNA from the 87DE region in the third chromosome of *Drosophila melanogaster*. This same region has been the subject of an extensive cytogenetic analysis by Hilliker *et al.* (1980). The 153 recessive lethal mutations that they collected divide into 21 complementation groups located within an interval (87D2–4 to 87E12–F1)

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‡ Abbreviations used: kb, 10^3 base-pairs.

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containing 23 to 26 polytene chromosome bands, as delineated in Bridges' (1941) map. The correspondence between the number of lethal complementation groups and the number of bands is striking, particularly as the number of bands is reduced to 21 ± 1 if one takes account of the dubious nature of Bridges' doublet bands (Beermann, 1962,1972; Berendes, 1970; Lefevre, 1976) and counts each of these as one rather than two bands (see Fig. 1). This region therefore provides one of several examples (Judd *et al.*, 1972; Hochman, 1973; Woodruff & Ashburner, 1979; Gausz *et al.*, 1979) indicating the existence of a one-to-one correlation between lethal complementation groups and the chromomeric units of polytene chromosomes. (A chromomeric unit equals the DNA sequence represented in a band plus an adjacent interband; Hogness *et al.* (1975).)

As will become apparent from the data presented here, the 315 kb walk includes 12 of the 21 complementation groups and covers 13 to 16 of the Bridges' bands (87D5–7 to 87E5, 6), or 13 ± 1 bands, if the doublets are counted as single bands. Two of these 12 complementation groups are represented by the well-studied loci,

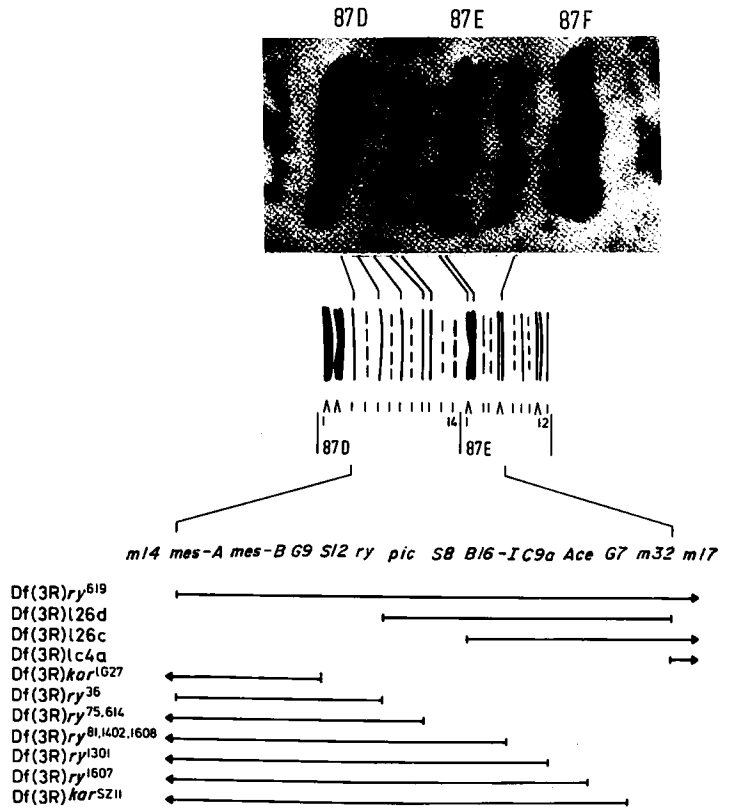

FIG. 1. Cytologic and cytogenetic maps of the 87DE region. The 87DE region was photographed by phase-contrast microscopy of *giant* chromosomes lightly stained with orcein. A micrograph of this region in a polytene chromosome has been mounted over the corresponding drawing from Bridges (1941). The cytogenetic map below this drawing has been adapted from Hilliker *et al.* (1980) and shows the lethal complementation groups and deficiencies pertinent to this paper.

rosy (Chovnick *et al.*, 1977) and *Ace* (Hall & Kankel, 1976), which code for xanthine dehydrogenase and acetylcholinesterase, respectively, and whose molecular cloning provided the initial incentive for our walk (Bender *et al.*, 1983). This convergence of molecular, cytogenetic and genetic information makes this region particularly suited to the study of chromosomal organization. In this paper we begin such a study by examining the relationship between the distribution of lethal complementation groups and chromomeric units at the molecular level.

Our experimental plan is divided into two parts. The first consists of mapping the DNA sequences of the chromomeric units, using for this purpose the *in situ* hybridization to polytene chromosomes of selected cloned segments from the set that defines the 315 kb region. The resulting molecular map not only indicates the position of the units, but also provides a measure of their lengths, which we find ranges from approximately 3 to 160 kb. This method for measuring the lengths of chromomeric units is distinguished from previous methods that rely on measurement of the total DNA mass per band–interband, and hence depend upon a particular model for the structure of polytene chromosomes (Beermann, 1972; Laird, 1980).

The second part of the plan consists of the molecular mapping of the 12 lethal complementation groups included within the region, using for this purpose the principle of deletion mapping. Hilliker *et al.* (1980) used this same principle to map these complementation groups genetically by determining which groups are included within each member of a set of overlapping deletions, or deficiencies in the lexicon of *Drosophila* geneticists. We in turn have mapped the breakpoints of these deficiencies on the 315 kb stretch of DNA. When combined with the genetic content of each deficiency, the positions of these deficiency breakpoints allow the construction of a molecular map of the 12 lethal complementation groups.

Alignment of the two molecular maps described above provides the desired comparison between lethal complementation groups and chromomeric units at the DNA sequence level. The result of that comparison is consistent with a one-to-one correlation between lethal complementation groups and chromomeric units within a region where the lengths of the units differ by almost two orders of magnitude.

2. Materials and Methods

(a) *Strains*

The recombinant λ phages containing segments of *D. melanogaster* DNA are described in the accompanying paper (Bender *et al.*, 1983). *D. melanogaster* strains are described in the text and by Hilliker *et al.* (1980); the deficiencies described by these authors were provided by A. Chovnick and J. Hall.

(b) *Methods*

With the exception of the *in situ* hybridization, all methods used here are described in the accompanying paper (Bender *et al.*, 1983). *In situ* hybridization was performed essentially as described by Pardue & Gall (1975). Squashes were pretreated for 30 min at 65°C in 2 × SSPE (SSPE is 0·15 M-NaCl, 10 mM-sodium phosphate, pH 7·0, 2 mM-EDTA)

and dehydrated before denaturation. RNase treatment was omitted. Hybridization buffer contained 4 × SSPE, 3 × Denhardt's (1966) solution, 0·1 mg sonicated salmon sperm DNA/ml, and 10^4 cts/min of nick-translated DNA/μl. Hybridizations were done at 65°C for 16 h. When a rapid result was preferred to high resolution, probe DNA was labeled with [^{125}I]dCTP instead of ^{3}H-labeled deoxynucleotides, and then 10% dextran sulfate, 1 mM-5-iodocytidine, and 0·2 mM-5-iodocytidine 5'-monophosphate were added to the hybridization mixture. Under these conditions, a 16 kb Dm segment produced as many as 15 grains/homologue per day.

3. Results

(a) *Molecular mapping of the chromomeric units*

The 87DE region is shown on a micrograph of the polytene chromosome from larval salivary glands (Fig. 1). The micrograph is aligned with the drawing of Bridges (1941), the basis for identification and numbering of bands, and with the genetic map of Hilliker *et al.* (1980). Our 315 kb walk extends from 87D5–7 to 87E5, 6 (this paper) and within this region, the large doublets 87E1, 2 and 87E5, 6 stand out. They are, however, typically observed as single bands in our preparations. While the smaller bands are less easily visible, we routinely recognize 87D5, D7, D9, D11 and D12 (Fig. 2(b)), which Bridges drew as solid lines. We also see 87D14 (Fig. 2(b)) and 87E4, while 87E3 is occasionally visible as a split from 87E1–2 (not shown). For the existence of the very faint bands 87D6, D8, D10 and D13, we rely on Bridges' expertise, and on the electron microscopy of Sorza (personal communication).

We have used the technique of *in situ* hybridization to identify the DNA sequences associated with these chromomeric units. The probes for this hybridization are cloned recombinant DNAs, each of which consists of a segment of *D. melanogaster* genomic DNA (Dm segment) inserted into a bacteriophage lambda vector (for details, see Bender *et al.*, 1983). The Dm segments in these experiments exhibit a mean length of 16·6 kb and range from 15·4 to 18·5 kb. We use the convention of Bender *et al.* (1983) of referring to a given recombinant phage and to its Dm segment by the number of that segment; thus, for example, the phage λcDm2851 and its Dm2851 segment are both abbreviated as 2851, except where they need to be distinguished. Recombinant DNAs chosen from various parts of the chromosomal walk were labeled with tritium by nick-translation (Rigby *et al.*, 1977) and hybridized *in situ* to polytene chromosomes from wild-type Oregon R or giant gt/gt^{x11} larvae. Some of the autoradiographs resulting from these experiments are given in Figure 2.

The results shown in Figure 2, or described in the text, are summarized in Figure 3, where the chromosomal positions of hybridization are aligned with the walk co-ordinates for the respective Dm segments (Bender *et al.*, 1983) to yield a low-resolution molecular map of the chromomeric units. Starting with 2851 (walk co-ordinates: $-204{\cdot}4 \pm 8{\cdot}3$ kb) at the left or proximal end of the walk, Figure 2(a) shows that the silver grains from its hybridization are spread over a large area covering 5 ± 1 faint or very faint bands (87D5–6 to 87D9–10). The grain pattern for the overlapping 2849 ($-193{\cdot}2 \pm 8{\cdot}1$ kb) was concentrated over the right end of

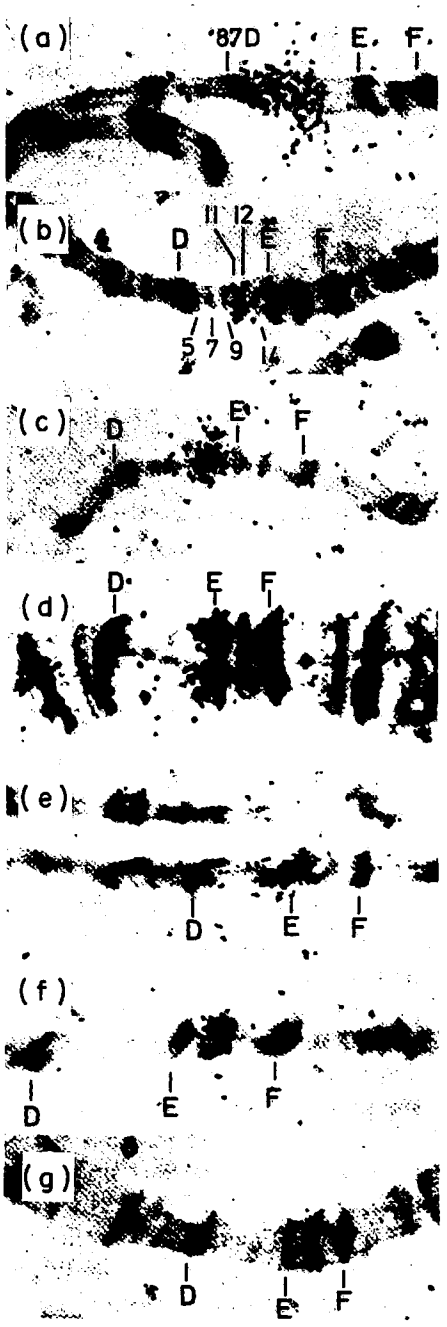

FIG. 2. *In situ* hybridization of DNA segments from the walk. Tritiated recombinant DNAs containing Dm segments from different parts of the walk were hybridized to polytene chromosomes from third instar salivary glands of Oregon-R or gt/gt^{x11} strains. The numbers of the segments (Bender *et al.*, 1983) responsible for the hybridization grains in the different micrographs are: (a) 2851, (b) 2841 and 2190, (c) 2837, (d) 2199, (e) 2198, (f) 2150 and (g) 2189.

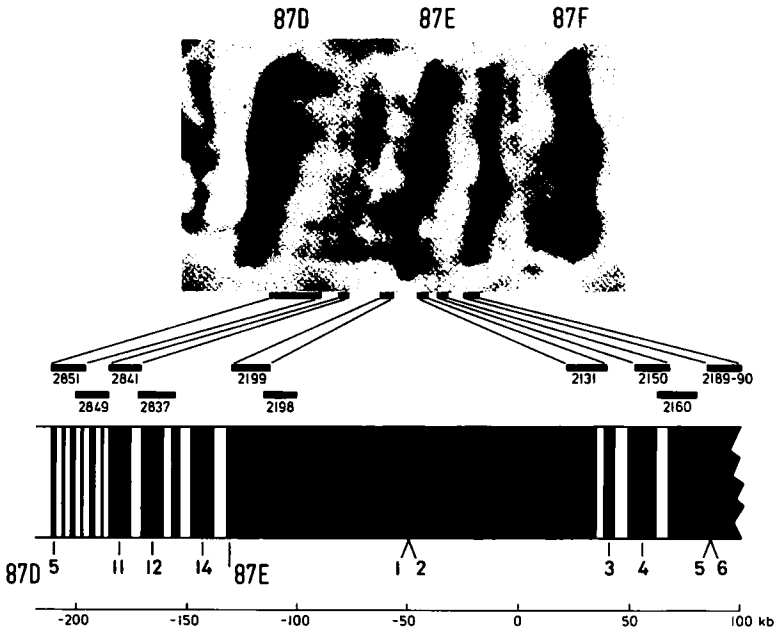

FIG. 3. Comparison of the cytologic and molecular maps of the chromomeric units. The filled bars immediately under the micrograph represent the areas of *in situ* hybridization resulting from the indicated Dm segment; whereas the numbered bars over the molecular map indicate the position of the Dm segments in the chromosomal DNA covered by the walk, whose co-ordinates (in kb) are given at the bottom of the Figure. The black and white vertical bars of the molecular map represent, respectively, the band and interband DNA of the chromomeric units. The relative widths (i.e. DNA sequence lengths) of the bands and interbands in the 87D5–14 and 87E3–4 regions of the map are arbitrary, whereas the positions of the ends of the 87E1, 2 and 87E5, 6 bands are not (see the text).

this region (not shown). By contrast, 2841 ($-177{\cdot}2 \pm 8{\cdot}5$ kb) produces grains centered on the single 87D11 band (Fig. 2(b); this chromosome was also hybridized to 2190, which is derived from the distal end of the walk and accounts for the band of grains within the 87E5, 6 doublet; see below). Continuing distally, Figure 2(c) shows that 2837 ($-163{\cdot}2 \pm 8{\cdot}4$ kb) produces grains spread over 87D12 and 87D13, while Figure 2(d) shows that 2199 ($-120{\cdot}1 \pm 9{\cdot}0$ kb) yields a rather sharp band of grains at the proximal edge of the large 87E1, 2 doublet band.

The distribution of silver grains produced by successively more distal segments indicates that this large doublet accounts for approximately half of the DNA covered by the walk. Thus, 2198 ($-107{\cdot}3 \pm 7{\cdot}7$ kb; Fig. 2(e)) and more distal segments produce grains clustered over 87E1, 2 until 2131 ($+31{\cdot}9 \pm 9{\cdot}2$ kb) is reached and the grains then extend out from the doublet to cover its distal edge (not shown). As shown in Figure 3, our interpretation of the grain patterns produced by the segments at or spanning the proximal and distal boundaries of 87E1, 2 is that most of 2199 is included within the band, while only half of 2131 is so included. We therefore estimate that the length of the DNA sequence encompassed by this large doublet band is approximately 160 kb (i.e. 8 kb more than the 152 kb that separates the centers of 2199 and 2131).

The sharp band of grains produced by 2150 ($+60{\cdot}1 \pm 7{\cdot}9$ kb) on the stretched chromosome shown in Figure 2(f) lies between 87E3 and the large 87E5, 6 doublet band. The proximal edge of this doublet is labeled by 2160 ($+71{\cdot}4 \pm 8{\cdot}0$ kb) (not shown), while the bands of grains produced by the more distal segments 2189 ($+92{\cdot}9 \pm 8{\cdot}3$ kb) and 2190 ($+94{\cdot}5 \pm 7{\cdot}8$ kb) lie within 87E5, 6, as shown in Figure 2(g) and (b), respectively. Since 2190 is the most distal segment of our walk (Bender *et al.*, 1983), the molecular map given in Figure 3 ends about 30 kb within the large 87E5, 6 band, preventing a determination of its full length. We reserve for the Discussion a consideration of the lengths of the fainter bands and of the level of resolution provided by this molecular map.

(b) *Molecular mapping of the lethal complementation groups*

As described in the Introduction, a low-resolution molecular map of the lethal complementation groups described by Hilliker *et al.* (1980) can be constructed by mapping on the DNA the breakpoints of the deficiencies that they used to order these groups on their genetic map. The technique that we used for mapping most of these breakpoints was again *in situ* hybridization, for it permits a test of whether a DNA segment is present or deleted in a defined region of the chromosome.

All the deficiencies of interest are homozygous lethal and had to be kept heterozygous with a non-deleted chromosome. Since homologues are paired in polytene chromosomes, it was difficult to determine whether a probe hybridized to the deficiency homologue when a signal was always seen over the wild-type homologue. This difficulty was circumvented by two approaches. Some deficiencies could be put opposite overlapping deficiencies when rescued by a duplication of the region on the fourth chromosome (Dp(3 : 4)ry^+, Lindsley & Grell, 1968). For example, we used a stock of Df(3R)126d over Df(3R)ry^{1607} (Fig. 1) carrying Dp(3 : 4)ry^+. When we hybridized with probes from regions clearly within the 126d deficiency, the presence or absence of grains at 87DE indicated whether the probe DNA was deleted in Df(3R)ry^{1607}. The duplication provided a positive internal control of hybridization (see Fig. 4(a) and (b) for examples). The second approach was to perform the hybridization on the deficiency-carrying chromosome kept over the chromosome balancer MKRS (*Tp* (3) *MKRS*, *M* (3) *S34 kar* ry^2 *Sb*) (Hilliker *et al.*, 1980). In this combination, the region of interest was asynapsed and we were able to distinguish easily between the deficiency-carrying arm and the balancer arm, which in this case provides the positive internal control of hybridization (Fig. 4(c) and (d)).

We have tested all the deficiencies described by Hilliker *et al.* (1980) that might exhibit breakpoints within our walk. In a typical set of experiments, probes originating from the proximal and distal ends of the walk were hybridized separately to chromosomes carrying one of the deficiencies. A negative result with one end of the walk and a positive one with the other showed that the deficiency breaks within the walk. When this was the case, the hybridizations were repeated with adjacent probes until the breakpoint was delimited by two overlapping or extremely close Dm segments. The results for all the deficiency breakpoints mapped by *in situ* hybridization are presented in Table 1. For each deficiency, we

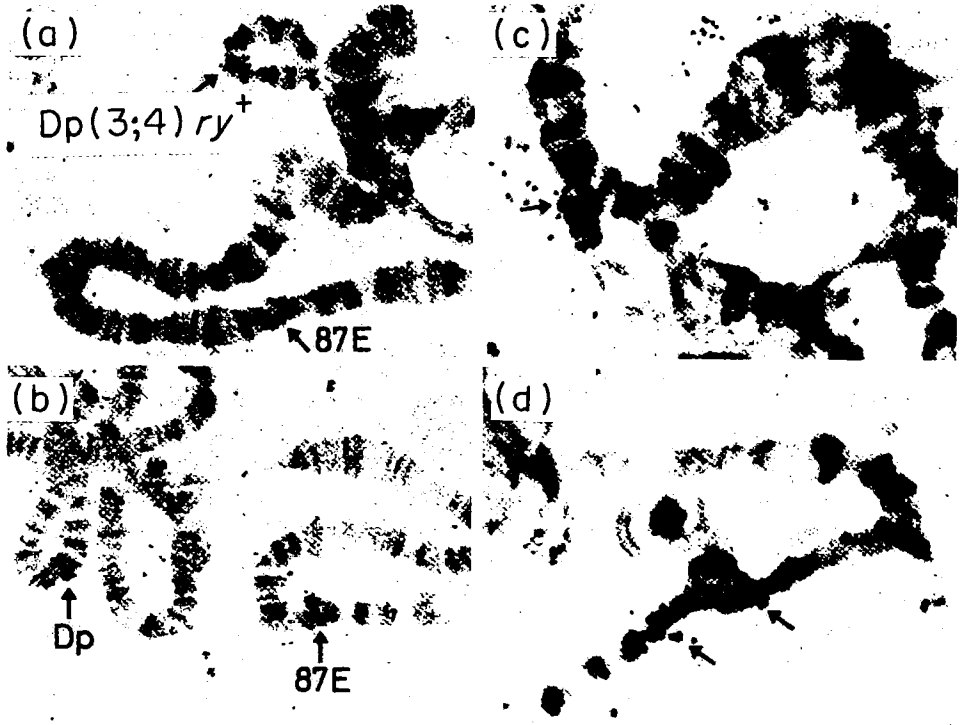

FIG. 4. Mapping deficiencies by *in situ* hybridization. *In situ* hybridizations patterns of: (a) 1923 (walk co-ordinates 17, 27) on Df(3R)ry^{1607}/Df(3R)126d + Dp(3 : 4)ry^+; (b) 1930 (33, 51·5) on the same chromosome combination as in (a); (c) 2849 (−201, −185) on Df(3R)ry^{36}/MKRS; and (d) 2851 (−213, −196) also on Df(3R)ry^{36}/MKRS.

list the "last" Dm segment giving a negative result (namely, within the deletion) and the "first" segment yielding a positive hybridization and consequently carrying DNA lying just outside of the deletion.

Figure 5 shows the positions of these breakpoints on the molecular map. A delimiting pair of "positive" and "negative" segments generally permits a localization of the breakpoint to within an interval of approximately 10 kb even though the average length of these segments is 16 kb. This results partly because many pairs consist of overlapping segments, and partly from a semi-quantitative reading of the observed hybridization. Thus, when no silver grains were observed, we assumed that less than 3 kb of the segment could be homologous to the chromosomal DNA; when the number of grains was strongly reduced compared to that on the non-deleted homologue, we assumed that less than half of the segment was homologous; and when the grains over the deficiency and the non-deleted chromosomes were quite similar, we assumed that more than half of the segment was homologous.

Figure 5 also shows the position of three breakpoints located by genomic restriction mapping (data not shown) rather than by *in situ* hybridization. In each case a new restriction fragment (fusion fragment) appeared in the place of

TABLE 1

Mapping of breakpoints by in situ *hybridization of cloned segments to deficiency-carrying chromosomes*

Deficiency breakpoint[a]	Closest positive[b] (walk co-ordinates)	Closest negative[b] (walk co-ordinates)	Balancer or heterozygous combination[c]
Df(3R)ry^{619}, proximal	2851 (−213, −196)	2849 (−201, −185)	MKRS
Df(3R)ry^{36}, proximal	2851 (−213, −196)	2849 (−201, −185)	MKRS
Df(3R) ry^{614}, distal	2821 (−141, −125)	2827 (−157, −139)	MKRS
Df(3R) ry^{75}. distal	2821 (−141, −125)	2827 (−157, −139)	MKRS
Df(3R)126c, proximal	2199 (−129, −112)	2198 (−115, −100)	Df126c/Df126d + Dpry^+
Df(3R)ry^{81}, distal	2198 (−115, −100)[d]	2199 (−129, −112)	MKRS
Df(3R)ry^{1402}, distal	2141 (−56, −39)	2165 (−77, −60)	MKRS
Df(3R)ry^{1608}, distal	2112 (−34, −17)	2140 (−57, −41)	MKRS
Df(3R)ry^{1301}, distal	1923 (17, 27)	2121 (−1, 20)	MKRS and Dfry^{1301}/Df126d + Dpry^+
Df(3R)ry^{1607}, distal	2131 (23, 41)	2121 (−1, 20)	MKRS
Df(3R)kar^{sz11}, distal[e]	2185 (78, 93)	2167 (66, 82)	TM3
Df(3R)1c4a, proximal	2192 (73, 88)	2189 (85, 101)	MRS
Df(3R)126d, distal	2189 (85, 101)	2192 (73, 88)	Df126c/Df126d + Dpry^+

[a] Hilliker *et al.* (1980).

[b] Recombinant phage number and co-ordinates according to Bender *et al.* (1983).

[c] MKRS, Hilliker *et al.* (1980); TM3 and Dpry^+ (Dpry^+ = Dp(3 : 4)ry^+), Lindsley & Grell (1968); MRS, is MKRS without *kar*.

[d] Strongly reduced signal.

[e] Gausz *et al.* (1979).

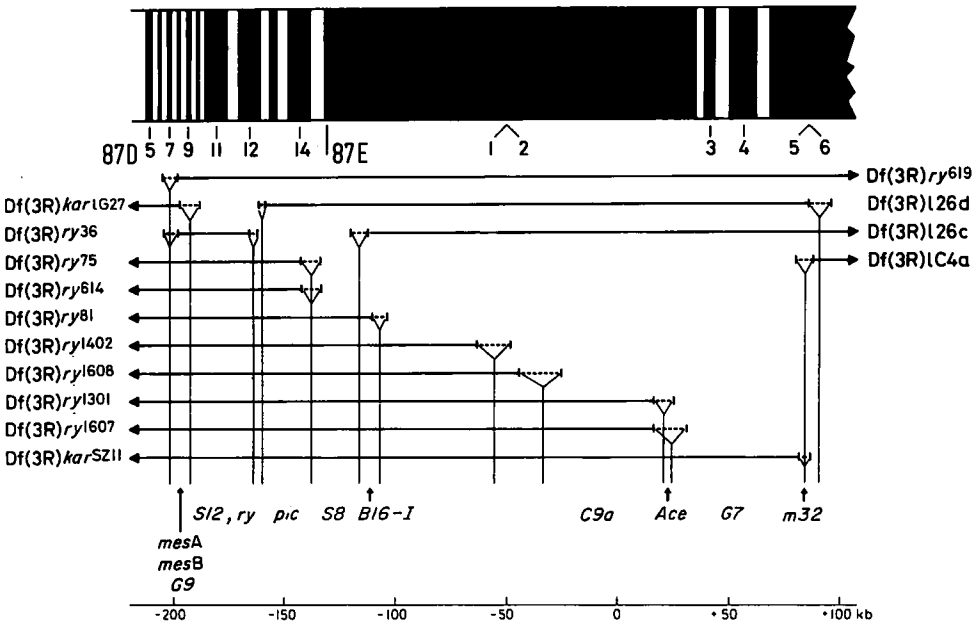

FIG. 5. Comparison of the molecular maps of chromomeric units and of lethal complementation groups. The map of the chromomeric units at the top is taken from Fig. 3. The filled horizontal lines below this map indicate the chromosomal DNA that is eliminated in the indicated deficiency, while the associated broken lines indicate the uncertainty of the breakpoint positions; arrowheads at the ends of these horizontal lines indicate that 1 of the 2 deficiency breakpoints lies outside the walk area. The positions of the 12 complementation groups are indicated below the horizontal lines, just above the map co-ordinates.

the wild-type fragment spanning the break. In addition, adjacent fragments within the deficiency disappeared while adjacent fragments outside the deficiency were present. This pattern is superimposed onto the wild-type pattern produced by the balancer chromosome MKRS in these heterozygous combinations. Using these criteria, we placed the distal break of Df(3R)kar^{1G27} in a 6·9 kb *Eco*RI fragment (walk co-ordinates: −191, −184), the distal break of Df(3R)ry^{36} in a 2·4 kb *Eco*RI fragment (−166, −163·5) and the proximal break of Df(3R)126d in a 3·0 kb *Hin*dIII fragment (−160·5, −157·5).

The positions of the 12 lethal complementation groups shown in Figure 5 are determined by the genetic content of the 14 deficiencies (Hilliker *et al.*, 1980) and are considered in the Discussion, as is the relationship between these positions and those of the chromomeric units, whose molecular map is included in Figure 5.

4. Discussion

(a) *DNA content and compaction of the chromomeric units*

The positions on the molecular map of the proximal and distal edges of 87E1, 2 are separated by 160 kb (Fig. 3). Bridges (1935,1941) characterized 87E1, 2 as a doublet and counted it as two bands. However, we typically observe it as a single

band, in keeping with a general difficulty in confirming the bipartite nature of Bridges doublets (Beermann, 1962,1972; Berendes, 1970; Lefevre, 1976). We therefore presume that the 160 kb bounded by the edges of 87E1, 2 lies within a single band; the largest covered by the walk. The DNA sequence length of this band is determined with reasonable accuracy because this length is much greater than the 15 kb confidence interval within which we can locate the edge of a band on the molecular map. This would also be the case for 87E5, 6, the second largest band in the walk area, were it not for the fact that the walk ends within this band, approximately 30 kb from its proximal edge. (A DNA sequence length of approximately 100 kb can be estimated for 87E5, 6 from its width relative to that of 87E1, 2.) By contrast, the DNA sequences of the remaining bands are too short to be measured individually at this level of resolution. We have therefore, divided this remainder into three regions and determined the mean DNA sequence length per chromomeric unit for each.

Consider first the 40 kb region bounded on the left by the distal edge of 87E1, 2 and on the right by the proximal edge of 87E5, 6 (Fig. 3). It includes two bands (87E3 and 4) and hence the mean DNA sequence length per chromomeric unit for this region is 20 ± 8 kb, where the ± 8 kb range derives from the uncertainty in the edge positions that define the region, and where we have ignored the effect of the extra interband as being small compared to this uncertainty. Proceeding proximally, the second region extends 58 kb from the proximal edge of 87E1, 2 to the proximal end of the 2841 segment, which we take to be the proximal edge of 87D11 (Fig. 3) because this 17 kb segment produces hybridization grains centered over this band (Fig. 2(b)). This region includes three readily visible bands (87D11, 12 and 14) and one very faint Bridges band (87D13) that we seldom, if ever, detect. The mean DNA sequence length for these four chromomeric units is therefore $14{\cdot}5 \pm 4$ kb. If we assume that the very faint 87D13 unit is ≤ 7 kb long (see below), then the mean for the three easily visible units is 17 ± 5 kb, very close to the mean for the two units of the first region.

The third region abuts the second and extends 27 kb to the proximal end of the walk. Consisting solely of faint and very faint bands, it allows an estimate of the DNA sequence lengths for the smallest units covered by the walk. The maximum number of bands included within this region is six: three faint bands that we can reliably detect (87D5, 7 and 9), and three very faint Bridges bands that we cannot (87D6, 8 and 10). The minimum number is four (87D7–10), given the above placement for the boundary between the third and second regions. Thus, while the hybridization grains produced by probes from the region appear to spread over all bands of the maximum set, none yields a sharp band of grains at the distal edge of the large 87D3, 4 doublet that would ensure the inclusion of this entire set within the 27 kb; the grain distribution does, however, ensure the inclusion of 87D7 and more distal bands. There are, therefore, a total of four to six chromomeric units within the 27 kb region, yielding a mean DNA sequence length of $5{\cdot}6 \pm 1{\cdot}1$ kb. Presumably, the chromomeric units with very faint bands have shorter lengths than those with faint bands, and hence are shorter than the above mean. If, for example, the mean lengths of these two classes differ by a factor of two, then the mean length for the very faint class would be $3{\cdot}7 \pm 0{\cdot}7$ kb.

Our measurements thus indicate that the DNA sequence length per unit varies from a maximum of approximately 160 kb to a minimum that may be as low as 3 kb and is not greater than 7 kb. They also yield a mean for the 13 ± 1 unit in the walk area of 30 ± 2 kb, providing that 87E5, 6, at the distal end of the walk, is included as a single band with an estimated length of 100 kb. These values are in reasonable agreement with those obtained previously from densitometric measurements of the total DNA mass of individual band–interband units in other regions of the polytene chromosomes. Thus, Rudkin's (1961) data on 60 individual band–interband units yield a mean DNA sequence length per chromomeric unit of 32 kb and a range of 7 to 92 kb (for the calculation of these values, see Hogness *et al.* (1975); see also Rudkin's (1972) review, where he notes the existence of larger units with lengths of a few hundred kb). Similarly, Beermann (1972), in reviewing these and other data, estimates a range from below 5 kb to approximately 100 kb.

There is a fundamental difference between the two methods for obtaining DNA sequence lengths, in that the densitometric method depends upon a particular model for the structure and replication of the banded arms of the polytene chromosomes, whereas our method does not. In the densitometric method, the measured DNA mass of a given band–interband is divided by the estimated number of haploid chromatic fibers making up that band–interband to obtain the desired DNA sequence length for the corresponding chromomeric unit. Since the number of chromatin fibers, or level of polyteny, is estimated by dividing the total DNA mass in the banded polytene arms by the haploid DNA mass for the equivalent euchromatic arms, it is assumed that the level of polyteny is the same for all bands and interbands in a given polytene nucleus. This assumption is equivalent to the commonly held model that the banded arms of polytene chromosomes are formed by proportionate DNA replication that yields a common level of polyteny. The alternative model of disproportionate replication, recently reintroduced by Laird (1980), provides for local variations in polyteny so that the number of haploid chromatin fibers in bands may differ from that in interbands and, indeed, may differ among bands and among interbands, as Laird supposes. Clearly, the densitometric method of obtaining DNA sequence lengths would give false values were the disproportionate replication model correct. By contrast, our method is independent of which model is valid. The general agreement between our results and those obtained by densitometric methods therefore favors the proportionate replication model. A better, more direct test of these opposing models is provided by the cloned segments from our walk, for they can be used as hybridization probes to determine the relative frequencies of occurrence of different sequences from the walk area in the total genomic DNA isolated from polytene nuclei (Spierer & Spierer, unpublished results).

Our method also has the attraction that the compaction of the chromosomal DNA (i.e. the packing ratio) calculated from its results is independent of the two models described above. The cytological distance covered by the 315 kb walk is 1·7 μm on micrographs of unstretched chromosomes (data not shown). This is to be compared with a value of 2·5 μm obtained from Bridges (1935) map of "moderately stretched salivary chromosomes", which is often used as a length

standard. We prefer to use our length measurements for calculating packing ratios because they should more closely approach those *in vivo*. (Ratios based on Bridges values would be approximately two-thirds of those calculated here.) The length of 315 kb of *B* DNA is 107 μm (107 μm = 315 kb/2·94 kb μm^{-1}); hence, the mean packing ratio for the DNA covered by the walk is 63 (63 = 107 μm/1·7 μm). This value compares well with the mean packing ratio of 57 for the DNA in all the polytene chromosome arms (calculated from Rudkin's (1972) determination that 78% of the 165,000 kb in the haploid genome is represented in the polytene arms, which have a combined length of 765 μm in the unstretched state; Bridges, 1942). A maximum packing ratio of approximately 180 is obtained for the 160 kb in the large 87E1, 2 band, which occupies 0·3 μm along the chromosome; and a minimum ratio of 23 is obtained for the 27 kb of DNA in the 0·4 μm region consisting of the faint and very faint 87D5–10 bands.

These values are to be compared with Beermann's (1972) estimate of approximately 100 and ≤ 5 for the mean packing ratios of bands and interbands, respectively, and with Laird's (1980) measurements, which yield means of 94 and 23, respectively. The difference between Beermann's and Laird's values for interband packing ratios is derived, for the most part, from different determinations of the fraction of total chromosomal mass or DNA present in the interbands; Beermann obtaining a maximum of 5%, and Laird a minimum of 26%. Our data cannot resolve this discrepancy, as the resolution of *in situ* hybridization with tritium-labeled probes is insufficient for determining DNA sequence lengths of interbands. Similarly, our lowest packing ratio of 23 for the region of faint and very faint bands, while suggesting that Laird's interband value is too high, is neither sufficiently inclusive nor accurate enough to insist that this is the case. More precise mapping of band/interband boundaries on the molecular map is required to answer this question. Progress in that direction has been made by Wu & Davidson (1981), who developed a new *in situ* hybridization method in which polytene chromosomes hybridized to DNA segments labeled with gold spheres are observed by transmission electron microscopy. Two segments from our walk were used in their experiments (2848 and 2188 near the proximal and distal ends, respectively; Bender *et al.*, 1983), and comparison of their results with ours indicates that a significant enhancement in resolution may be achieved by their method.

(b) *Correspondence between the positions of chromomeric and genetic units on the molecular map*

Hilliker *et al.* (1980) used 15 deficiencies to order the 21 lethal complementation groups that they defined in the course of saturating the 87D2–4 to 87E12–F1 region with lethal loci. All but one of these deficiencies exhibit at least one breakpoint within the 215 kb walk area, and we have located all of these breakpoints on the molecular map shown in Figure 5. The proximal boundary of this breakpoint distribution is at walk co-ordinate -201 ± 3 kb, only 12 ± 3 kb from the proximal end of the walk, and the distal boundary is at $+90 \pm 5$ kb, only 12 ± 5 kb from the distal end of the walk. Twelve of the complementation groups

are located within the 291 ± 8 kb between these boundaries, and the positions of these are delimited by the positions of the deficiency breakpoints that separate individual complementation groups, or clusters of two to three groups, from their immediate neighbors. Superimposition of the molecular map for the chromomeric units onto this map of complementation groups allows the desired comparison between the two distributions (Fig. 5).

We first note the close correspondence between the numbers of chromomeric and genetic units in the whole region, and then show that this correspondence holds for its four subregions. Given that the region lacks the proximal 12 kb of the walk and that the mean DNA sequence length per chromomeric unit is 6 kb for the faint and very faint units in this area, we estimate that the number of chromomeric units in the whole region is 2 less than the 13 ± 1 units in the walk area, or 11 ± 1; in good agreement with the 12 complementation groups in the region.

For the same reason, we estimate that the number of chromomeric units in the subregion extending 15 kb from the proximal end of breakpoint distribution to the proximal edges of 87D11 is 3 ± 1, or 2 less than the 5 ± 1 faint or very faint units within the proximal 27 kb of the walk. Three complementation groups (*mesA*, *mesB* and *G9*) map within this subregion, which may also include *S12*. (Although *S12* is not separated from *rosy* (*ry*) by a breakpoint, it maps proximal to *ry* by recombination; Hilliker *et al.* (1980).) Proceeding distally, the next subregion consists of four chromeric units (87D11–14) and contains 4 ± 1 complementation groups, depending upon whether *S12* and/or *B16–I* are included. The next subregion is the 160 kb 87E1, 2 band, which possibly contains only one complementation group (*C9a*), and no more than three, depending upon whether *B16–I* and/or *Ace* are included. Finally, the 60 kb subregion that extends from the distal edge of 87E1, 2 into 87E5, 6, to the distal end of the breakpoint distribution includes DNA sequences from two to three complementation groups, depending upon whether *Ace* is included. In summary, consistent one-to-one ratios for the numbers of genetic and chromomeric units can be assigned to each of the four subdivisions. Thus, a one-to-one ratio is continuously obtained as the size of the chromosomal region yielding that ratio is progressively decreased from the 87D2–4 to 87E12–F1 region with its 21 lethal complementation groups (see the Introduction), through the 291 kb region with its 12 groups, to its four subregions. Extrapolation, therefore, provides a strong presumption that when the resolution is increased further to the level of single units, the same ratio will hold.

Better resolution in the mapping of the complementation groups could be achieved by increasing the number of deficiencies examined so as to decrease the map distance between their breakpoints. However, this method is limited by the possibility that a deficiency could produce effects beyond its endpoints; i.e. a deficiency could inactivate a gene without including its coding sequence. A better method for increasing resolution is to use the DNA fragments from the walk as hybridization probes for the detection of homologous RNAs, thereby mapping the transcription units associated with the complementation groups to any desired resolution down to the base-pair.

This method has the added advantage of allowing the detection of genes that

would be missed by conventional saturation screens for lethal and visible mutants, such as that used to define the lethal complementation groups mapped here. For example, these screens would miss mutations in repeated genes (e.g. the histone genes; Lifton *et al.*, 1978) and in single-copy genes whose products are not essential for viability or normal morphology (e.g. the *Sgs-4* gene; Muskavitch & Hogness, 1980,1982). Although the more sophisticated mutational screens used by Young & Judd (1978) suggest that the number of genes missed by conventional screens is low, we have little information about the relative numbers of genes that are detectable by their transcripts but not by conventional screens. For instance, we have almost no basis for predicting whether the 73 ± 13 kb region within the 87E1, 2 band that is bounded by the distal breakpoints of Df(3R)ry^{81} and Df(3R)ry^{1608} (Fig. 5) is genetically silent with respect to transcription, as it is with respect to lethal complementation groups. It is an important question because its answer will determine whether the one-to-one correspondence between genetic and chromomeric units is limited to a particular class of genes. Some of us have therefore extended the analysis of our walk to determine the distribution of its transcription units (Hall *et al.* (1983)).

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REFERENCES

Beermann, W. (1962). In *Handbuch der Protoplasmaforschung*, vol. 4d, pp. 1–161, Springer, Vienna.

Beermann, W. (1972). In *Results and Problems in Cell Differentiation*, (Beermann, W., ed.), vol. 4, pp. 1–33, Springer-Verlag, Berlin.

Bender, W., Spierer, P. & Hogness, D. S. (1983). *J. Mol. Biol.* **168**, 17–33.

Berendes, H. D. (1970). *Chromosoma*, **29**, 118–130.

Bridges, C. B. (1935). *J. Hered.* **26**, 60–64.

Bridges, P. N. (1941). *J. Hered.* **32**, 299–300.

Bridges, P. N. (1942). *J. Hered*, **33**, 403–408.

Chovnick, A., Gelbart, W. & McCarron, M. (1977). *Cell*, **11**, 1–10.

Denhardt, D. T. (1966). *Biochem. Biophys. Res. Commun.* **23**, 641–646.

Gausz, J., Bencze, G., Gyurkovics, H., Ashburner, M., Ish-Horowicz, D. & Holden, J. J. (1979). *Genetics*, **93**, 917–934.

Hall, J. C. & Kankel, D. R. (1976). *Genetics*, **83**, 517–535.

Hall, L. M. C., Mason, P. J. & Spierer, P. (1983). *J. Mol. Biol.* In the press.

Hilliker, A. J., Clark, S. H., Chovnik, A. & Gelbart, W. M. (1980). *Genetics*, **95**, 95–110.

Hochman, B. (1973). *Cold Spring Harbor Symp. Quant. Biol.* **38**, 581–589.

Hogness, D. S., Wensink, P. C., Glover, D. M., White, R. L., Finnegan, D. J. & Donelson, J. E. (1975). In *The Eukaryote Chromosome* (Peacock, W. J. & Brock, R. D., eds), pp. 1–33, Australian National University Press, Canberra.

Judd, B. H., Shen, M. W. & Kaufman, T. C. (1972). *Genetics*, **71**, 139–156.

Laird, C. D. (1980). *Cell*, **22**, 769–874.

Lefevre, G. (1976). In *Genetics and Biology of* Drosophila (Ashburner, M. & Novitski, E., eds), vol. 1a, pp. 31–66, Academic Press, New York.

Lifton, R. P., Goldberg, M. L., Karp, R. W. & Hogness, D. S. (1978). *Cold Spring Harbor Symp. Quant. Biol.* **38**, 581–589.

Lindsley, D. L. & Grell, E. (1968). In *Genetic Variations of* Drosophila melanogaster, publication 627, Carnegie Institute of Washington.
Muskavitch, M. A. T. & Hogness, D. S. (1980). *Proc. Nat. Acad. Sci., U.S.A.* **77**, 7362–7366.
Muskavitch, M. A. T. & Hogness, D. S. (1982). *Cell*, **29**, 1041–1051.
Pardue, M. L. & Gall, J. G. (1975). In *Methods in Cell Biology* (Prescott, D., ed.), vol. 10, pp. 1–16, Academic Press, New York.
Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977). *J. Mol. Biol.* **98**, 503–517.
Rudkin, G. T. (1961). *Microchem. J. Symp. Ser.* **1**, 261–276.
Rudkin, G. T. (1972). In *Results and Problems in Cell Differentiation* (Beermann, W., ed.), vol. 4, pp. 59–85, Springer-Verlag, Berlin.
Woodruff, R. C. & Ashburner, M. (1979). *Genetics*, **92**, 133–149.
Wu, M. & Davidson, N. (1981). *Proc. Nat. Acad. Sci., U.S.A.* **78**, 7059–7063.
Young, M. W. & Judd, B. H. (1978). *Genetics*, **88**, 723–742.

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