Molecular and Cytogenetical Characterization of the 10A1-2 Band and Adjoining Region in the *Drosophila melanogaster* Polytene *X* Chromosome

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ABSTRACT

Some 300 kb of DNA from the 9F12–10A7 X chromosome region (seven bands) uncovered by $Df(1)v^{L3}$ were cloned and 31 break points of chromosome rearrangements within the region were mapped. Positions of 12 genes found earlier in genetic saturation experiments, transcripts and *P* element-induced mutations were located on the physical map using either chromosome rearrangements or Southern blot hybridizations. Data on the position of the break points, genes and polytene chromosome bands allow the following conclusions to be made. (1) The size of the bands in the region varies between 4 kb (10A6 and 7) and 183–195 kb (10A1-2). The compaction ratio of DNA in bands varies from 8–36 (10A6 + 7) to 151–161 (10A1-2). Therefore, fine and thick bands appear to have different kinds of DNP packaging. (2) The bands differ in genetic content. Fine bands contain from one to three genes. In contrast, the 10A1-2 band contains three genes and at least six transcribed DNA fragments. (3) Comparison of genetic and physical maps shows that in this region 0.01 centiMorgan corresponds to 3.3 kb of DNA.

NUMEROUS ideas about the organization of polytene chromosome bands and interbands have appeared [see ZHIMULEV *et al.* (1981a) for details] since the proposal of KOSTOFF (1930) that bands or "discs may represent the actual packets in which inherited characters are passed from generation to generation." For a long time the the most popular hypothesis was that one gene was located within one band [see for review, LEFEVRE (1974)].

At least three lines of evidence appeared in the 1980s suggesting that this hypothesis was incorrect. First, genetic saturation of small chromosomal regions showed that the distribution of mutations (mainly lethals) was not uniform along a chromosome. There were both "empty" regions and regions where the number of essential genes was more than the number of bands (reviews: Zhimulev 1981a, b, 1987; LEFEVRE and WATKINS 1986).

Second, after the cloning and molecular characterization of numerous polytene chromosome regions it was shown that the frequency of gene distribution (one gene per 1–7 kb of DNA) was much higher than that of bands (one band per 30 kb) (GILBERT *et al.* 1984; ZHIMULEV and BELYAEVA 1985; GAUSZ *et al.* 1986; WRIGHT 1987). As a result, it was proposed that bands be classified according to their genetic content (ZHIMULEV and BELYAEVA 1985): (i) *polygenic bands* containing moderately repeated histone genes, genes of 5S, 18S and 28S ribosomal RNA; (ii) *oligogenic bands* containing several genes in a band (10A1-2, 9F12, 37C1-2, 87E1-2, 89E1-4) (ZHIMULEV *et al.* 1981b; WRIGHT 1987; LAWRENCE 1992); (iii) *simple bands*, containing only one gene (bands of the 3AB region) (JUDD *et al.* 1972); and (iv) *silent bands* in which genes were either not found or found to occupy a very small part of the band (3C2-3, 10B1-3) (SORSA *et al.* 1973; ZHIMULEV *et al.* 1987).

Third, total polysomal RNAs isolated from either late third-instar larvae or cell lines of *Drosophila melanogaster* correspond to about 17,440 diverse molecules of average size 1,250 nucleotides. This number of genes exceeds the number of bands by approximately a factor of three, if bands are counted according to Bridges' map (ZIMMERMANN *et al.* 1980) or by a factor of five if one counts the "doublets" of Bridges as "singlets." In the latter case, the number of bands in Drosophila does not exceed 3500 (ZHIMULEV 1992).

Genetic analysis combined with a molecular characterization of bands could help our understanding of their organization. The 9F12–10A7 region of the *D. melanogaster X* chromosome could give important advantages for this kind of analysis. Using electron microscopic mapping seven bands were found in the region uncovered by $Df(1)v^{L3}$. The region was saturated with different kinds of mutations: visibles, lethals, male and female steriles. Several types of bands with different genetic content were described: the 9F12 band contains three genes, *fliG*, *fs(1)BP* and *l(1)9Fe*. The 10A1-2 band could be divided by chromosome rearrangement break points into four zones: silent DNA on its distal side, *v* and *l(1)10Aa* in the distal middle part, a silent zone in the proximal middle part and the *sev* gene in the proximal part of the band (ZHIMULEV *et al.* 1987). In regions with

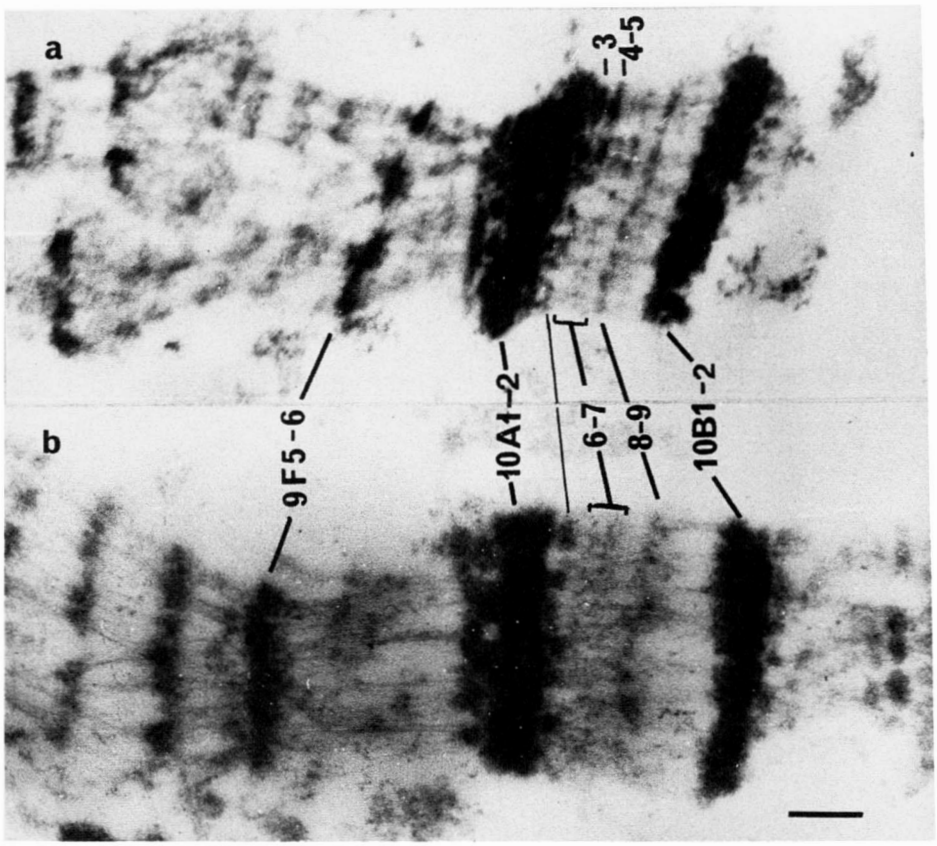

FIGURE 1.—EM mapping of the 10A region in a normal chromosome (a) and *Df(1)sev*^{P1} chromosome (b). Scale represents 1 μm.

TABLE 1

List of new chromosome rearrangements

Chromosome	Description	Source
Df(1)v^{L7}	*Df(1)9E1-3;10A3*	G. LEFEVRE, JR.[a]
In(1)w^{mhr500}	*In(1)3C1;10A1-2*	G. REUTER[b]
Df(1)sev^{P1}	*Df(1)10A1-2;10A1-2*	U. BANERJEE[c]

[a] G. LEFEVRE (1969). Cytology revised in the present study (see text for explanation).
[b] G. REUTER, unpublished.
[c] BANERJEE *et al.* (1987).

TABLE 2

Progeny of matings of *Df/FM6* females (carrying the indicated deficiency) to *1(1)10Ac/y*$^{+}$*Yv*$^{+}$ males

Deficiency	*FM6/1(1)Ac*	*Df/1(1)10Ac*
Df(1)sev^{P1}	96	97
Df(1)v^{L7}	413	430

thin bands (9F13, 10A3, 10A4-5, 10A6 and 10A7) a ratio similar to one gene:one band was found.

The data we have obtained permit the most complete analysis to date comparing the genetic and molecular organization of individual bands as chromosome units. In this study the results of our cloning, molecular and cytogenetic characterization of the region, are presented.

MATERIALS AND METHODS

Drosophila chromosomes and stocks: New chromosomes used are listed in Table 1. Descriptions of others can be found in ZHIMULEV *et al.* (1987) or in LINDSLEY and ZIMM (1992).

TABLE 3

Progeny of matings of *Df/FM6* females (carrying the indicated deficiency) to *slm/y*$^{+}$*Yv*$^{+}$ or *slm*^{G102}*/y*$^{+}$*Yv*$^{+}$ males

	FM6/slm	Df/slm	FM6/slm^{G102}	Df//slmG102
Df(1)sev^{P1}	147	72	75	31
	84	77	26	27
	66	85	39	65
Df(1)v^{64f29}	80	84		
	137	142		
	92	87		
Df(1)v^{L3}	165	1	25	0
	135	69	19	9
	85	111	15	26
Df(1)v^{L1}	228	5	52	0
	101	54	36	2
	72	126	17	31
Df(1)v^{L7}	151	133		
	127	136		
	108	117		

Numbers of imago eclosing during the first, second and third days of eclosing are shown in three lines.

Cytology: Salivary gland chromosomes squashed in lacto-acetic orcein were analyzed using phase contrast microscopy according to standard procedures. The methods used for electon microscopy are described elsewhere (ZHIMULEV *et al.* 1981b). *In situ* hybridizations were performed essentially as described in GALL and PARDUE (1971) except that 10% dextran sulfate was added to the hybridization solutions.

Microcloning: The microdissection of the 10A1-2 band, all the treatment of chromosome pieces and the cloning the DNA fragments were performed as described (PIRROTTA *et al.* 1983).

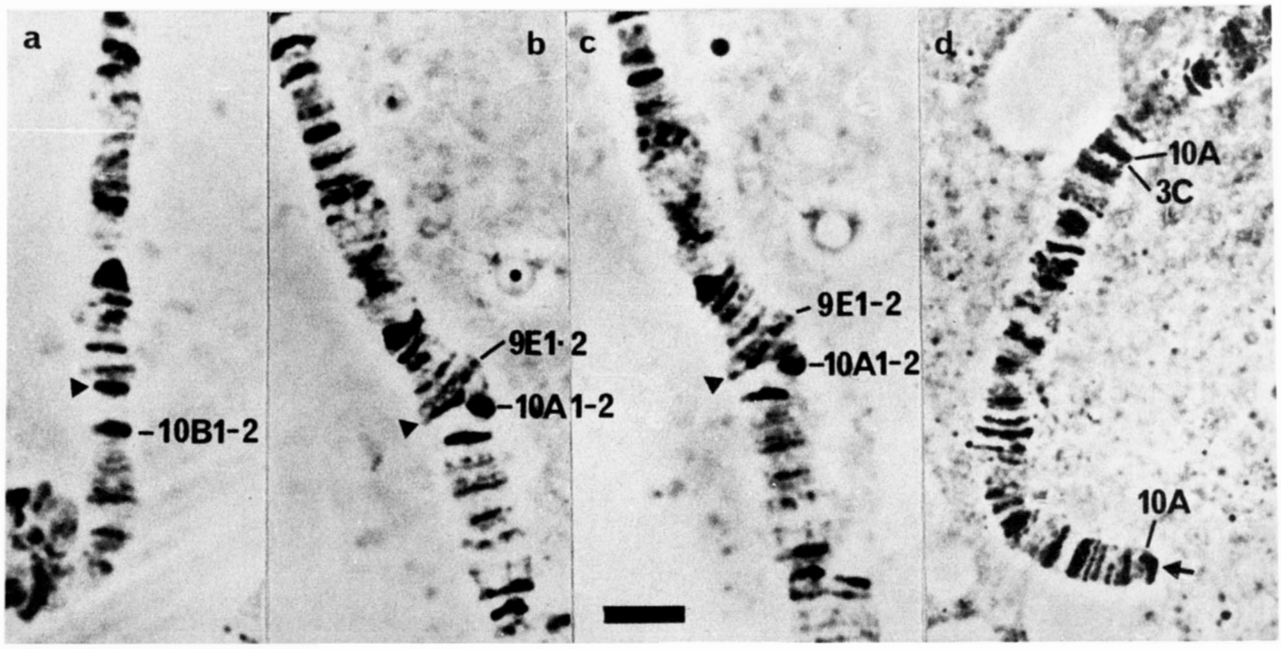

FIGURE 2.—Light microscope mapping of $Df(1)v^{l7}$ (a–c) and $In(1)w^{mhr500}$ (d). Arrowhead indicates the 9E1-3 region in the $Df(1)v^{L7}$ chromosome. The arrow in (d) shows the position of attachment of pericentric heterochromatin. Scale represents 5 μm.

DNA cloning: A chromosome walk was started using the clones resulting from the microdissection. All walking was done according to BENDER *et al.* (1983) with some modifications. Phage DNA purification, its labeling by nick-translation and Southern blot hybridization were done using methods described by MANIATIS *et al.* (1982). Drosophila genomic DNA was purified as described by BENDER *et al.* (1983). Genomic clones were screened from: (1) a Canton-S library (MANIATIS *et al.* 1978), (2) an Oregon-R library cloned into λEMBL4 (gift of F. KAFATOS), (3) an Oregon-RC library cloned into λL47.1 (gift of N. A. TCHURIKOV) and (4) a library originating from a stock isogenized for second chromosome (*dp, cl, cn, bw*) cloned into λDASH II (gift of W. GELBART). Endonucleases *Hin*dIII, *Sal*I, *Bam*HI and *Eco*RI were used for construction of restriction maps. Some of the clones used (pvSE3.5; cED3.1; 1A, 2E, 3B, 4G, 5D, 5F, 11H) were previously mapped within the 10A1-2 band and were kindly provided by R. G. TEARLE, E. HAFEN, S. BENZER and U. BANERJEE, respectively.

Northern blot analysis: Poly(A)$^+$ RNA from various embryonic stages was prepared using a Poly(A)Tract mRNA Isolation System (Promega). Gel electrophoresis and transfer of RNA was performed as in MANIATIS *et al.* (1982). The probe used for hybridization was a 1.3-kb *Eco*RI fragment of cDNA clone 901 that lacked plasmid sequences labeled with a Random Primed DNA Labelling Kit (Boehringer Mannheim). After 3 days of autoradiographic exposure at −70° with an intensifying screen, the blot was stripped and hybridized with rp 49 probe (O'CONNEL and ROSBASH 1984). The length of autoradiographic exposure with rp 49 probe was 30 min. RNA size standards used were 0.24–9.5 kb.

RESULTS

Mapping of new chromosome rearrangements: *$Df(1)sev^{P1}$:* This deficiency removes about 50 kb of DNA in the proximal part of the 10A1-2 band and uncovers the *sev* gene (BANERJEE *et al.* 1987). It is invisible at the level of the light microscope. At the electron microscope (EM) level, two rather thick bands, 10A3 and 10A4-5, are clearly seen proximal to the 10A1-2 band (Figures 1a and 3a) in normal chromosomes. However, in the $Df(1)sev^{P1}$ chromosome only the 10A4-5 band proximal to 10A1-2 is seen (Figure 1b). There are several possible explanations for our inability to see band 10A3: (i) the deficiency removes only the 10A1-2/10A3 interband region, (ii) both the proximal part of 10A1-2 and the distal part of 10A3 including the interband are deleted and (iii) interband 10A1-2/10A3 and part or all of the 10A3 band are deleted. In all these cases the material of the 10A1-2 and 10A3 bands is joined to form a single band visible on Figure 1b as the thick 10A1-2 band. We conclude that even with the EM we are at best able to map rearrangement break points with a precision of not more than one interband ± edges of neighboring bands, or one band ± neighboring interbands [see ZHIMULEV *et al.* (1981a) for discussion].

Within the region of the 10A3–10A4-5 band three genes, *ms(1)BP*, *slm* and *l(1)10Ac* have been located (ZHIMULEV *et al.* 1987). Genetic experiments show (Table 2) that $Df(1)sev^{P1}$ does not uncover *l(1)10Ac*. Mutations of the *slm* locus result in forming of small slim body in adult flies and a strong delay of imago eclosion during the first two days (ZHIMULEV *et al.* 1987) when they are uncovered by $Df(1)v^{L3}$ or $Df(1)v^{L1}$. $Df(1)v^{64f29}$, which covers *slm*, was used as a control (Table 3). In all combinations of *slm* mutations with deficiences showing the delay in eclosion heterozygous *Df/slm* females had small slim bodies. This phenotype was expressed in very small degrees in $Df(1)sev^{P1}/slm$ heterozygotes (Table 3). Interaction of *ms(1)BP* with $Df(1)sev^{P1}$ was not tested because of loss of the *ms(1)BP*.

$In(1)w^{mhr500}$: This inversion was obtained by screening for non-variegated revertants of $In(1)w^{m4}$ following irradiation (REUTER *et al.* 1985). As a result, the 10A1-2 band is broken and its biggest (distal) part is brought to

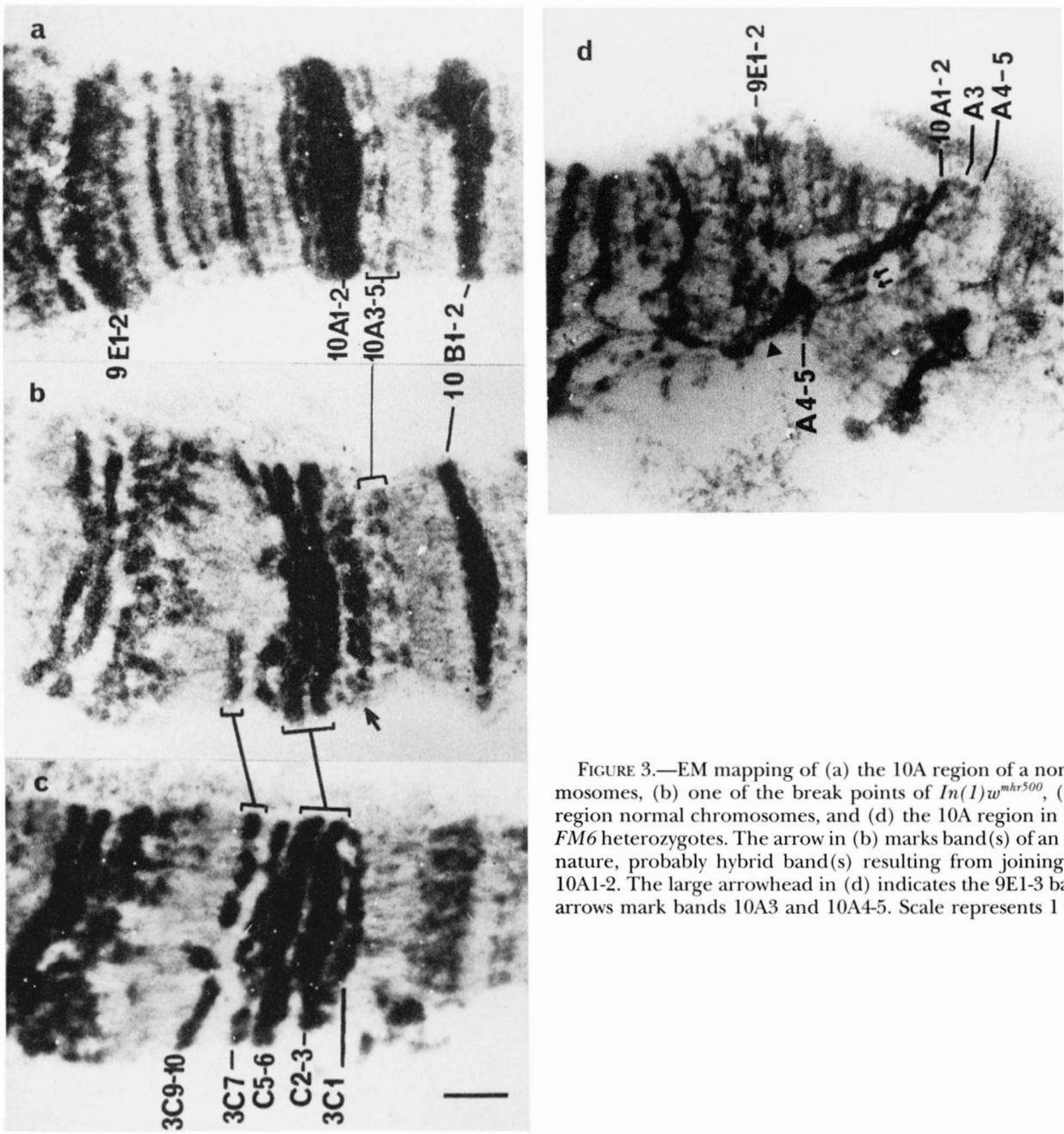

FIGURE 3.—EM mapping of (a) the 10A region of a normal chromosomes, (b) one of the break points of $In(1)w^{mhr500}$, (c) the 3C region normal chromosomes, and (d) the 10A region in $Df(1)v^{L7}/FM6$ heterozygotes. The arrow in (b) marks band(s) of an indefinite nature, probably hybrid band(s) resulting from joining 3C1 and 10A1-2. The large arrowhead in (d) indicates the 9E1-3 band, small arrows mark bands 10A3 and 10A4-5. Scale represents 1 μm.

the vicinity of pericentric heterochromatin (Figure 2d). The smaller (proximal) part of 10A1-2 joins bands of the 3C region (Figure 2d). Examination of the rearranged chromosome with EM reveals that bands of both 10A3–10B1-2 (Figure 3b) and 3C2-3–10A1-2 (Figure 3c) are clearly visible with unidentifiable material present between these regions (indicated by arrow on Figure 3b). In particular note the gray dotted band or two bands in both regions not present in normal chromosomes. This band(s) most likely is derived from parts of 3C1 and the most proximal part of 10A1-2 which join or almost join into a single band (there is some duality in this band (see Figure 3b). Therefore, $In(1)w^{mhr500}$ has a break point distal to the proximal edge of the 10A1-2 band. Genetically the inversion break point maps within the limits of the *sev* gene, because heterozygotes $In(1)w^{mhr500}/sev$ manifest the *sev* mutant phenotype–six rhabdomeres in an ommatidia (not shown).

$Df(1)v^{L7}$: According to Lefevre (1969) this deficiency deletes the region between 9E1-3 and the middle part of band 10A1-2. Based on his data the same limits were given in our previous paper. However, in the course of mapping the proximal break point of this deficiency on the molecular map (see below) we found that it removed the whole 10A1-2 band. It was shown before (ZHIMULEV *et al.* 1987) that cytologically *T(1;Y)B105* breaks the *X* chromosome in very proximal part of 10A1-2 band, $Df(1)v^{64f29}$ and $Df(1)ras\text{-}v^{17Cc8}$ remove almost whole band 10A1-2. Nev-

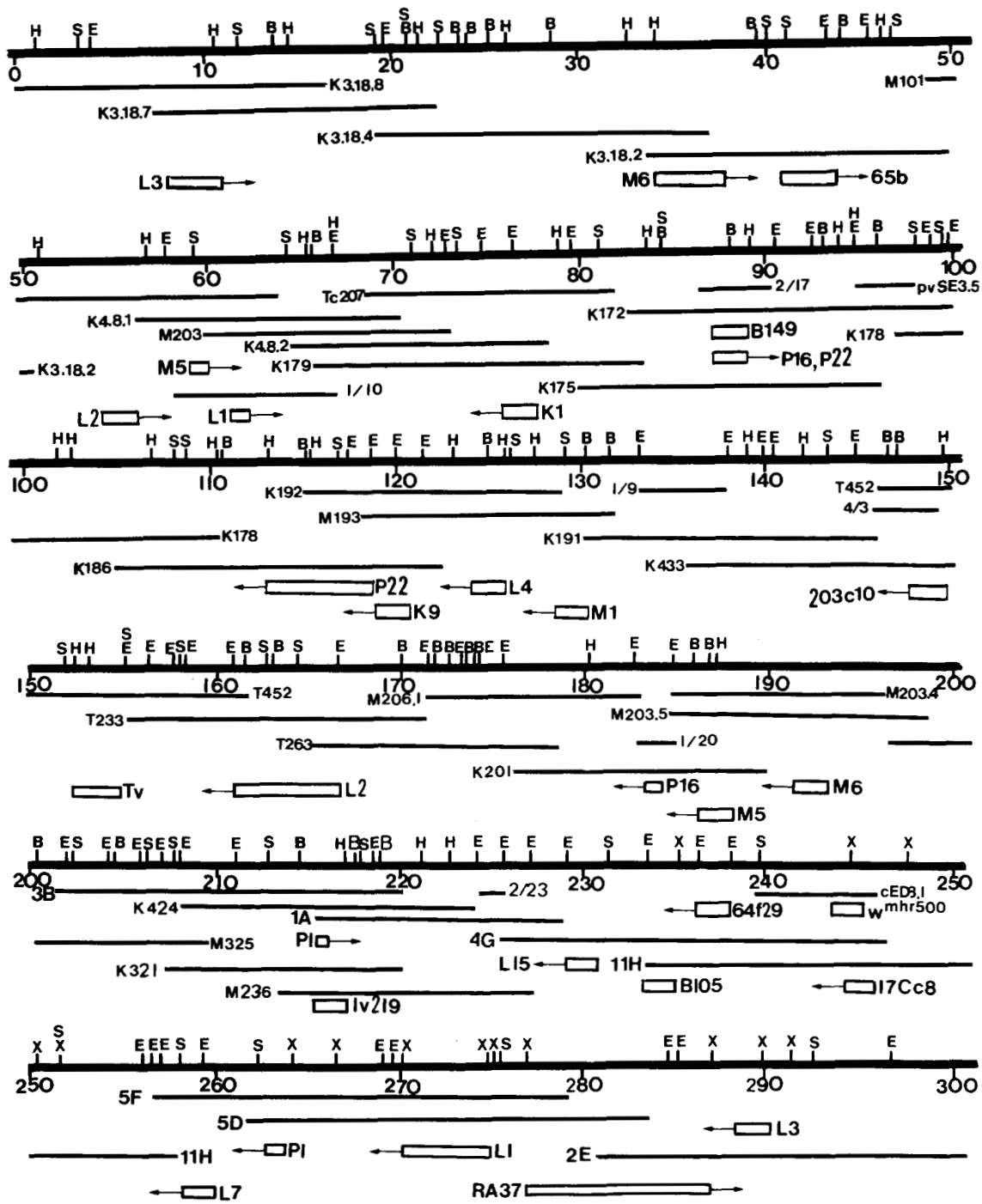

FIGURE 4.—The map of the cloned genomic region 9F12–10A6-7 of the *D. melanogaster X* chromosome. The thick line is restriction map with distances given in kilobase pairs. Restriction sites are shown by symbols: E, *Eco*RI; H, *Hin*dIII; B, *Bam*HI; S, *Sal*I; X, *Xho*I. Overlapping DNA inserts of recombinant phages are indicated below the restriction map. M, K, Tc and T series from MANIATIS, KAFATOS, TCHURIKOV and GELBART libraries, respectively. Positions from 230 to 300 follow the restriction map by BANERJEE *et al.* (1987). Open bars indicate the uncertainty of the rearrangement break point positions. Arrows indicate direction of deficiencies. Short clones, 1/10, etc., are microdissection clones used for start points of walking.

ertheless, the position of the break point of $Df(1)v^{L7}$ is proximal to all these rearrangements on the molecular map. Therefore, cytology of this rearrangement was reinvestigated.

As follows from Figure 2a in a chromosome with the deficiency distal to 10B1-2 there is a rather big band (marked with an arrowhead on Figure 2a), which was erroneously thought to be 10A1-2. In heterozygous larvae at some focal planes in the light microscope this band contacts 10A1-2 of the normal homolog. The rest of the band therefore can mimic the proximal part of the 10A1-2 band. However, at other focal planes the

TABLE 4

Localization of chromosome rearrangement breakpoints on physical map of the 9F12–10A7 region

Break points, distal (D) or proximal (P)	Clone	Mapping	Position on the map (kb)
Df(1)v^{L3} (D)	3.18.7	Most distal 2.4-kb *Eco*RI-*Hin*dIII-fragment	8–10.5
Df(1)v^{M6} (D)	3.18.4	Most proximal 4-kb *Eco*RI-*Hin*dIII-fragment	34–38
Df(1)v^{65b} (D)	3.18.2	*In situ*: clone is broken in the middle	41–44
Df(1)v^{L2} (D)	3.18.2	*In situ*: clone is not removed	54–56
	M 101	*In situ*: clone is broken in the middle	
	4.8.1	*In situ*: clone is removed	
Df(1)v^{M5} (D)	1/10	*In situ*: deletion removes about 80%	59–60
	M203	*In situ*: clone is removed	
Df(1)v^{L1} (D)	1/10	*In situ*: deletion removes about ⅔ of the clone	61–62
	M203	*In situ*: deletion removes about 80% of the clone	
Df(1)sbr^{K1} (P) *T(1;Y)B149* *Df(1)v^{P16}* (D)	Tc207	*In situ*: deletion removes about 60% of the clone	75.5–77.5
Df(1)v^{P22} (D)	2/17	*In situ*: rearrangement translocates about ⅓ of the clone	88–90
Df(1)v^{P22} (P)	K192	*In situ*: clone is not removed	113–119
Df(1)sbr^{K9} (P)	K192	*In situ*: deletion removes about 50% of the clone	119–121
	M193	*In situ*: deletion removes not more than 10% of the clone	
Df(1)v^{L4} (P)	K192	*In situ*: deletion removes about 70% of the clone	124–126
	M193	*In situ*: about half of the clone is removed	
Df(1)v^{M1} (P)	M193	*In situ*: deletion removes about 80% of the clone	128.5–130.5
Df(1)203C10 (P)	K433	*In situ*: deletion removes about 80% of the clone	148–150
	4/3	*In situ*: the same	
T(1;3)v	T452	*In situ*: 50–70% of the clone is in proximal element	151.5–155
Df(1)v^{L2} (P)	T452	*In situ*: deletion removes about 90% of the clone	161–166
	T233	*In situ*: about 40% of the clone is removed	
Df(1)v^{P16} (P)	1/20	*In situ*: clone is broken in the middle part	183–184
	K201	*In situ*: the same	
Df(1)v^{M5} (P)	K201	*In situ*: deletion removes about 75% of the clone	186–188
Df(1)v^{M6} (P)	M203.5	*In situ*: clone is broken in the middle part	191–193
	M203.4	*In situ*: deletion removes about 60% of the clone	
Df(1)sev^{P1} (P)	3B	According to BANERJEE *et al.* (1987): in the middle of 3.5 kb *Xho*I-*Sal*GI-fragment	215–216
T(1;2)lv219 (P)	M236	*In situ*: 90% of the clone is in proximal element	214–216
	K424	*In situ*: clone is broken in the middle part	
Df(1)v^{L15} (P)	4G	*In situ*: deletion removes not more than 20% of the clone	229–231
	2/23	*In situ*: clone is removed	
T(1;Y)B105	4G	*In situ*: clone is broken in the middle part	233–235
	11H	*In situ*: clone is in proximal element	
Df(1)v^{64j29} (P)	11H	According to HAFEN *et al.* (1987): in 1.4 kb. *Eco*RI-fragment distally to clone cED3.1 (71.3–72.7), which corresponds to position of 1.5-kb *Eco*RI fragment	236.5–237
In(1)w^{mhr500}	11H	*In situ*: clone is broken in the middle part	243.5–245.5
Df(1)ras-v^{17Cc8}	11H	According to HAFEN *et al.* (1987): in 3.0 kb. *Xho*I-fragment of cED3.1 clone (67–64), which corresponds to position of 3 kb *Xho*I fragment	244–246
Df(1)v^{L7} (P)	11H	*In situ*: deletion removes clone	258–260
	5F	*In situ*: deletion removes about 15% of clone	
Df(1)sev^{P1} (P)	5D, 5F	According to BANERJEE *et al.* (1987): within 2-kb *Sal*GI-*Xho*I fragment	262.5–264.5
Df(1)v^{L1} (P)	5D	Within 4.5-kb *Xho*I fragment	270–274.5
		In situ: deletion removes about half of the clone	
	5F	*In situ*: deletion removes about 80% of the clone	
Df(1)RA37 (D)	2E	*In situ*: deletion removes the clone	277–285
Df(1)v^{L3} (P)	2E	*In situ*: deletion removes about half of the clone	287.5–289.5

same band can be seen to contact 9E1-3 and consequently is homologous to the latter (Figure 2c). With the EM it can be seen that, proximal to the thick band marked with an arrowhead on Figure 3d, there is only a thin band, 10A4-5, so the 10A3 band is either removed by deficiency or joins 9E1-3 bands. This means that the proximal break point of *Df(1)v^{L7}* is located either within the limits of 10A3 or on the border between 10A3 and its distal interband.

Genetically *Df(1)v^{L7}* does not uncover either *l(1)10Ac* or *slm* (Tables 2 and 3) but does uncover *sev* and more distal genes.

Cloning the region: A library of microdissection clones was used for cloning the region. First, about 30 microdissection clones were mapped against a set of chromosome rearrangements dividing the region into several zones (*Df(1)v^{L1}*, *T(1;Y)B149*, *Df(1)v^{L4}* and *Df(1)v^{64j29}*). Next, those that were located between break points were used as probes for walking.

Approximately 230 kb of contiguous DNA was isolated as genomic clones and mapped using the restriction enzymes *Eco*RI, *Hin*dIII, *Sal*I and *Bam*HI (Figure 4). Data on an additional 70 kb from the

proximal part of this region were taken from a paper by BANERJEE *et al.* (1987).

Chromosome rearrangement break points were mapped either by Southern genomic blots or by *in situ* hybridization to polytene chromosomes. Salivary glands from female larvae heterozygous for a given rearrangement and *FM6* balancer chromosome or hemizygous male larvae with a duplication of the 10A region translocated onto the *Y* chromosome (Dpy^+Yv^+) were used for the polytene squashes. The ratio of the silver grains on chromosomes with and without the rearrangements was used to determine if part of a clone was removed by the rearrangement. This approach permits the mapping of break points with a precision of 10–20% (BELYAEVA *et al.* 1987). Data on break point localizations are shown in Table 4.

About half of the DNA clones in the region are unique because they only hybridize to one region of the polytene chromosomes. DNA fragments in positions 3–10, 86–99, 112–137, 164–166, 202–215 and 258–261 kb contain repeated sequences (to be published elsewhere).

Mapping of the genes: Several *P* element-induced mutations in the *l(1)9Fg*, *l(1)10Ac*, *l(1)10Ad* and *l(1)10Ae* loci were isolated previously (POKHOLKOVA *et al.* 1991). Using Southern blot hybridization, six mutations in the *l(1)10Ac* locus were mapped within the 4.5-kb *Xho*I fragment of clone 5D in position 270–275. Three mutations of *l(1)10Ad* are located in the 8.5-kb *Xho*I fragment in position 276.5–285 kb; *l(1)D41*, the only *P* element-induced representative of 1(1)10Ae, maps between 287 and 289.5 kb. For the first two loci, cDNA clones were isolated from an ovarian cDNA library probed with the 4.5-kb *Xho*I and the 6.5-kb *Xho*I-*Sal*I fragments of clone 5D.

We were particularly interested in determining if sequences in the middle of band 10A1-2, apparently "silent" in the genetic saturation screen, are also transcriptionally inactive. It had been shown that the 1/9 microdissection clone (133–138 kb on the physical map) shows a strong signal in dot-blot hybridization with labeled poly(A)$^+$ RNA from the whole third instar larvae (KOKOZA *et al.* 1990). Using this clone as a probe, a cDNA clone (901) was isolated from a prepupal cDNA library. To analyze further the expression of this putative gene we performed Northern blot hybridization with RNA from different stages of Drosophila embryogenesis. Poly(A)$^+$ RNA from embryos 0–4, 4–8, 1–16, 15–19, 19–23 hr after egg laying was size-separated on an agarose gel containing formaldehyde, transferred to a membrane and probed with the 1.3-kb *Eco*RI fragment of cDNA clone 901. One 2.4-kb transcript was found to be expressed between 8 and 15 hr after egg laying (Figure 5). The signal is undetectable prior to 8 hr. On the longer exposures a very faint 2.4-kb band could be seen in the 15–19-hr lane (not shown).

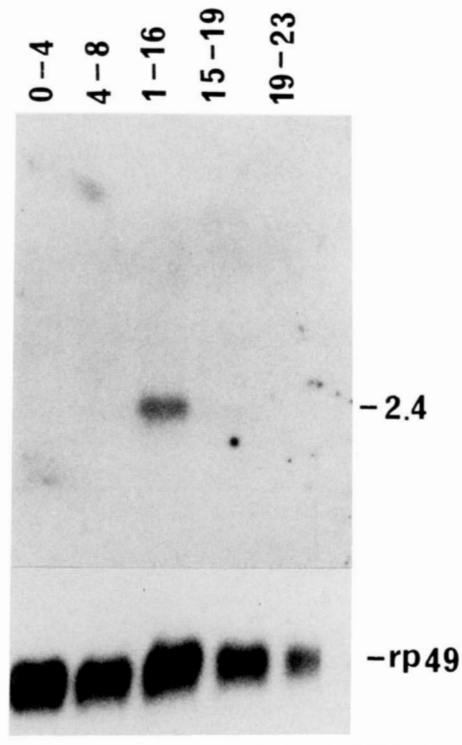

FIGURE 5.—Northern blot analysis of cDNA clone 901 expression. A Northern blot with poly(A)$^+$ RNA from various stages of Drosophila embryogenesis is shown (numbers refer to hours of development after fertilization). One transcript, 2.4 kb, is detected with 901 cDNA probe. To check loading and integrity of RNA the same blot was hybridized with control probe rp49 (lower panel).

DISCUSSION

We have further characterized the 9F12–10A7 region using a combination of mapping: (1) new rearrangement break points, (2) *P* element-induced mutations, (3) clones and (4) some transcripts, all with respect to the mutations that have been identified in this region. The distribution of the bands, genes and transcribed DNA on the physical map of DNA are shown in Figure 6. The whole region comprises almost 300 kilobase pairs. The 9F12 band is located between the break points of $Df(1)v^{L3}$ and $Df(1)v^{M6}$ deficiences break points. Since the break points are mapped both at the cytological and molecular level with some uncertainty, a minimum size of the band is estimated between the most proximal possible break point location of the $Df(1)v^{L3}$ and the most distal of the $Df(1)v^{M6}$ (shown in black on Figure 6), that is 23.5 kb. The maximal size of the band is estimated as 34 kb, between the most distal possible break position of $Df(1)v^{L3}$ and the most proximal site of $Df(1)v^{65b}$. In the latter case, we subtracted 2 kb of the proposed interband length. White intervals in Figure 6 correspond to the uncertainty in the limits of two neighboring bands plus the interband between them. The sizes of all bands in the region are shown in Table 5. The 10A6 and 10A7 bands are not separated by chromosome rearrangements and their sizes, including the interband between

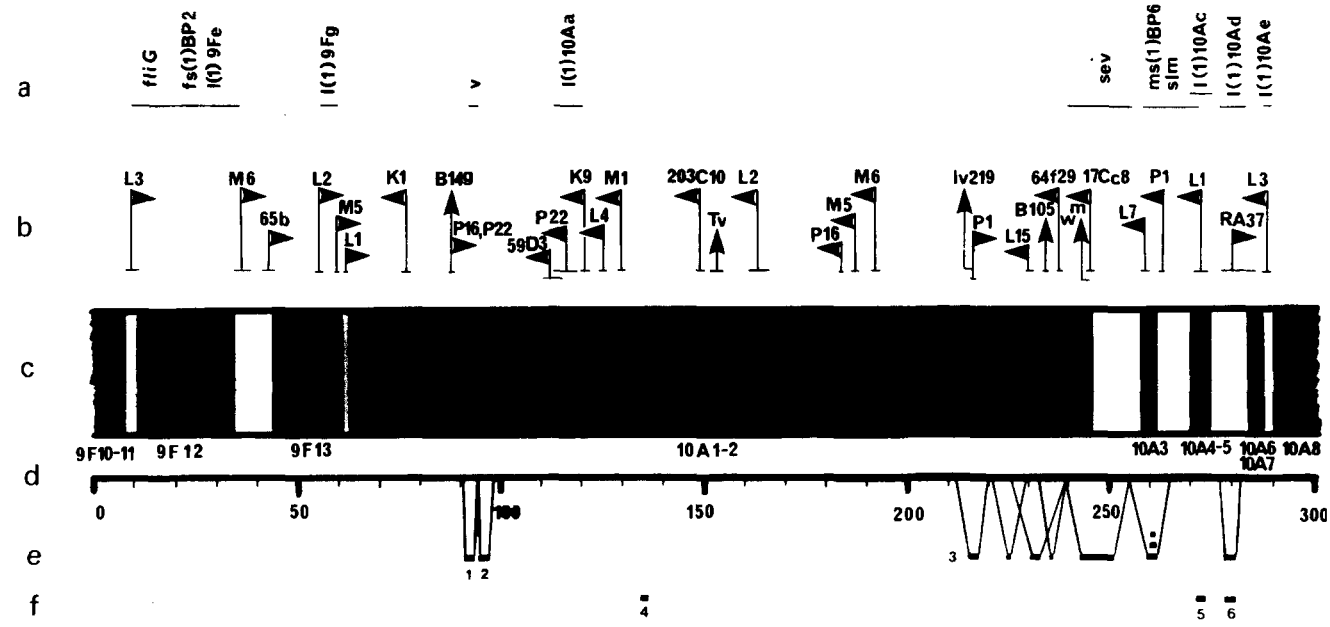

FIGURE 6.—Molecular and cytogenetic map of the 9F12–10A7 region of the *D. melanogaster X* chromosome. (a) Genetic loci, found in saturation experiments and positioned between chromosome rearrangement break points (ZHIMULEV *et al.* 1987; this paper). (b) Mapping of chromosome rearrangement break points on the physical map. Arrowheads show the direction of deficiencies. Designation of chromosome rearrangements: L3, $Df(1)v^{L3}$, M6, $Df(1)v^{M6}$; 65b, $Df(1)v^{65b}$; L2, $Df(1)v^{L2}$; M5, $Df(1)v^{M5}$; L1, $Df(1)v^{L1}$; K1, $Df(1)sbr^{K1}$; B149, *T(1;Y)B149*; P16, $Df(1)v^{P16}$; P22, $Df(1)v^{P22}$ ($Df(1)v^{P16}$ and $Df(1)v^{P22}$ were obtained in *T(1;Y)B149*, with identical distal breaks in all three rearrangements); 59D3, *Df(1)59D3*; K9, $Df(1)sbr^{K9}$; L4, $Df(1)v^{L4}$; M1, $Df(1)v^{M1}$; 203C10, *Df(1)203C10*; Tv, *T(1;3)v*; lv219, *T(1;2)lv219*; P1, $Df(1)sev^{P1}$; L7 = $Df(1)v^{L7}$; L15, $Df(1)v^{L15}$; B105, *T(1;Y)B105*; 64f29, $Df(1)v^{64f29}$; w^m, $In(1)w^{mhr500}$; 17Cc8, $Df(1)ras\text{-}v^{17Cc8}$; RA37, *Df(1)RA37* [see Zhimulev (1981b,c, 1987) for details]. (c) Banding pattern of the region. Sizes of the bands are given in scale, according to location of the chromosome break points both on cytological (c) and physical (d) maps. The DNA sequences which are exactly located within the bands are shown in black, uncertainties in the mapping of bands margins and neighboring interbands are shown in white. (d) Physical map with distances in kilobase pairs. (e and f) Location of fragments coding for transcripts (e) and cDNA clones (f); 1 and 2, *vermilion* and 2-kb transcripts, respectively (SEARLES and VOELKER 1986; WALKER *et al.* 1986); 3–9, transcripts (HAFEN *et al.* 1987; BANERJEE *et al.*, 1987); 4, 901 cDNA clone; 5 and 6, cDNA clones, found in ovarian library probed with 4.5 *Xho*I (5) and 6.5 *Xho*I-*Sal*I (6) fragments of clone 5D.

TABLE 5

Sizes and DNA compaction (ratio) of the bands in the 9F12–10A7 region

Bands and interbands (IB)	Length along chromosome axis[a] (mcM) (*a*)	Location between break points: minimal/maximal	Physical sizes (in kb) minimal/maximal	Length of DNA (mcM), minimal/maximal (*b*)	Compaction ratio (*b*/*a*), minimal/maximal
9F12	0.92 + 0.009	*L3*(D)-*M6*(D)[b]/*L3*(D)-*65b*(D)	23.5/34.4	7.99/11.56	86/125
IB	0.048 + 0.009				
9F13	0.086 + 0.011	*65b*(D)-*L1*(D)/*M6*(D)-*L1*(D)	17/27	5.78/9.18	67/106
IB	0.043 + 0.009				
10A1–2	0.410 + 0.046	*L1*(D)-*17Cc8*(P)/*L1*(D)-*L7*(P)	183/195	62.22/66.3	151/161
IB	0.095 + 0.019				
10A3	0.078 + 0.006	*L7*(P)-*P1*(P)/*17Cc8*(P)-*L1*(P)	4/22	1.36/7.48	17/95
IB	0.065 + 0.008				
10A4–5	0.092 + 0.007	Limits of *L1*(P) mapping/*P1*(P)-*RA37*(D)	4.5/21	1.53/7.14	17/77
IB	0.144 + 0.023				
10A6	0.057 + 0.006				
IB	0.045 + 0.008	Both bands are between			
10A7	0.064 + 0.005	*RA37*(D)-*L3*(P)/*L1*(P)-*L3*(P)	3/13	1.02/4.42	8/36

[a] Estimated on electron microscope sections of 50 polytene chromosomes (V. F. SEMESHIN, unpublished).
[b] D, P, distal and proximal break points, respectively.

them, are given as a sum in the Table 5, that is about 10 kb. Taking into account that the bands have similar sizes when examined with the EM (see Figure 1) and that interbands according to calculations contain about 1 kb of DNA [see for review BEERMANN (1972) and ZHIMULEV (1992)], one may estimate the DNA length of these bands as 4 kb. Therefore the sizes of the bands in the region vary between 4 kb and 183–195 kb.

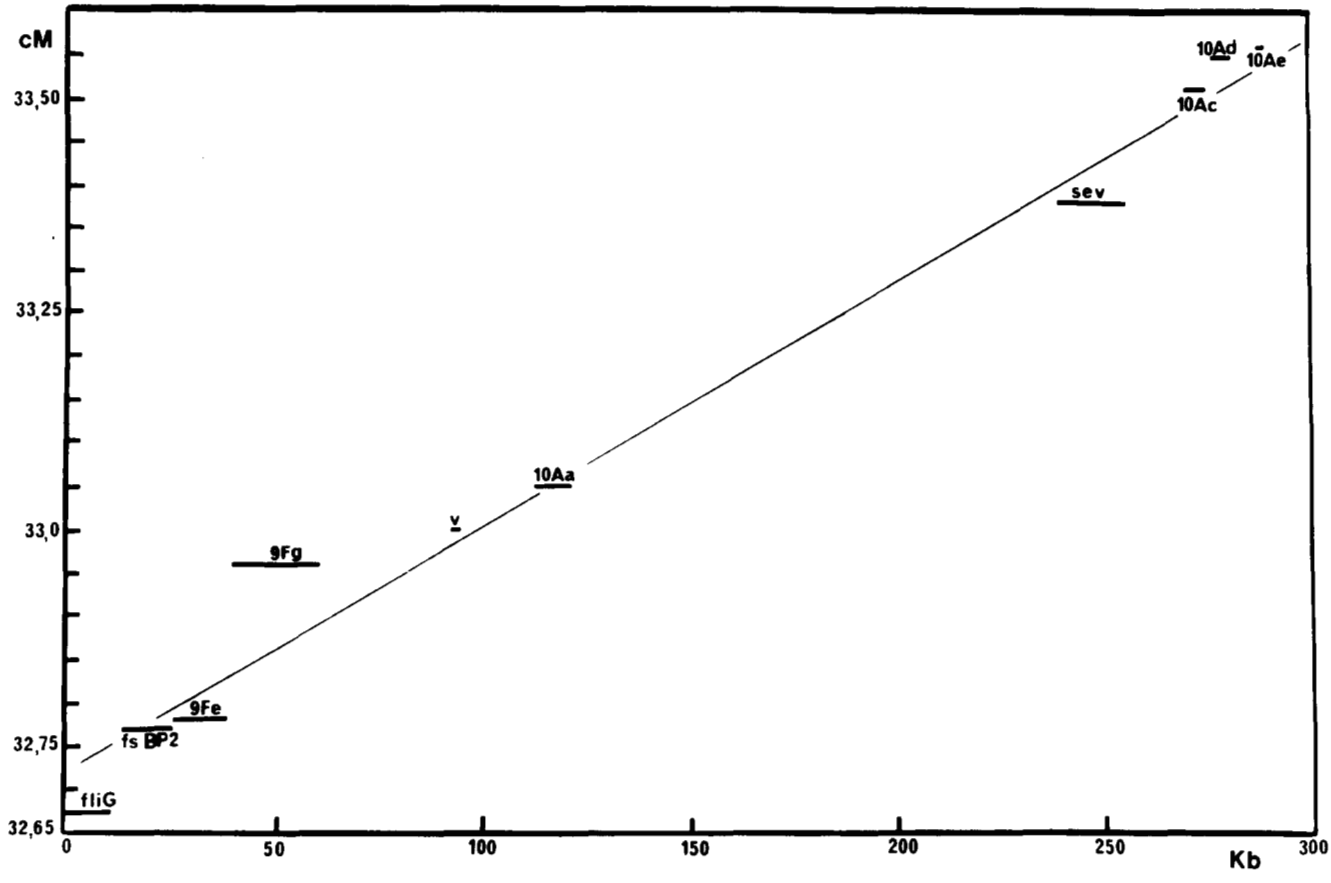

FIGURE 7.—Correspondence between positions of the genes on the molecular (abscissa) and genetic (ordinate) maps. Distances are given in kilobase pairs (abscissa) and map units (ordinate).

Similar data were obtained for the 87D5-6–87D9-10 region where small bands contained less than 7 kb and the finest even 3 kb. The biggest band at 87E1-2 contains about 160 kb of sequence (SPIERER 1984).

At the EM level the length of bands along the chromosome axis varies between 0.057 and 0.41 μm. Less variability exists in the interbands, between 0.043 and 0.144 μm (Table 5). Therefore, the DNA compaction ratio is about 8–36 for the finest bands 10A6 and 10A7, and 151–161 for the 10A1-2 bands (Table 5). Similar variability between fine and thick bands was found in the 87DE region (SPIERER 1984). The compaction ratio of the small bands obtained in transformation experiments is about 30–54 (SEMESHIN *et al.* 1989). These estimates are noticeably lower then that found for the Balbiani ring 2 chromomere (380) in *Chironomus tentans* (DERKSEN *et al.* 1980).

All of the data show that the compaction ratio of small and big bands is different. For small ones it corresponds to the nucleomeric level [20–56, see ZHIMULEV (1992) for review]. Thick bands show compaction ratios more than 100, which means that DNP fibers in thick bands are packed more tightly by at least a factor of two than in fine bands.

Because the nucleomere fiber is composed only of DNA and histone molecules, other kinds of protein molecules might be involved in compaction of thick bands. Morphologically thick and fine bands are different as well: the former usually look dark and very dense under phase contrast, while the latter are diffuse to some extent, do not stain as heavily and look gray under phase contrast.

The distribution of genes within the cloned interval is uneven. Regions of small bands are populated more densely. The 9F12 band contains three genes in 23.5–34 kb, the 9F13 band contains one gene in 17–27 kb, bands 10A3 and 10A4-5 contain three genes in 17–36 kb, and bands 10A6 and 10A7 contain two genes in 3–13 kb (Figure 6).

At the same time only three genes, *v*, *sev* and *l(1)10Aa* were found in genetic saturation experiments within 183–195 kb of the 10A1-2 band (Figure 6). We did not perform a systematic search for transcripts within the interval cloned. Nevertheless some data on the new DNA fragments coding for RNA were obtained either from our experiments or from the literature.

In a 4-kb *Eco*RI fragment next to *vermilion* there is a transcribed sequence, representing a gene other than *vermilion* since the size of the transcript is different and it is expresed in all *vermilion* mutants (SEARLES and VOELKER 1986; WALKER *et al.* 1986).

At approximately position 135 kb on physical map (Figure 6) there is another transcribed sequence that results in a 2.4-kb RNA (see Figure 5). This RNA might

correspond to a new gene located in the middle part of the 10A1-2 band, unidentified in previous genetic screens.

Several transcribed fragments were found in 30 kb near the proximal margin of the 10A1-2 band. Beside the DNA in position 239–255 kb coding for the 8.7-kb RNA of the *sev* gene (BANERJEE *et al.* 1987; BOWTELL *et al.* 1988), there are at least 4 more DNA fragments that hybridize to RNA (see Figure 6). Therefore, the part of the 10A1-2 band between *l(1)Aa* and *sev*, previously called silent DNA (ZHIMULEV *et al.* 1981c, 1987), comprises at least five transcribed fragments.

The very precise data on the genetic positions of loci in the 9F12–10A7 interval obtained in previous work (ZHIMULEV *et al.* 1987) and now at the molecular level permit us to estimate the relationship of the molecular and recombinational maps of the genes (Figure 7). The whole distance between *fliG* and *l(1)10Ae* is as long as 0.89 cM; on the molecular map it is about 290 kb. The positions of the genes fit very well to a straight line, which corresponds to about 0.01 cM per 3.3 kb of DNA. This figure is similar to the 3.8 kb/0.01 cM (RUDKIN 1965; LEFEVRE 1971) found for the interval between *w* and *f*.

In previous reports (ZHIMULEV *et al.*, 1981c, 1983) it was suggested that the "silent DNA zone" between *l(1)Aa* and *sev* genes was a hot spot of chromosome rearrangement break points. However, after estimations of the physical length of the band and exact mapping 10A1-2 it is clear that rearrangements are distributed rather evenly throughout the band (see Figure 6).

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