GENETIC CHARACTERIZATION OF THE REGION BETWEEN 86F1,2 AND 87B15 ON CHROMOSOME 3 OF *DROSOPHILA MELANOGASTER*

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Manuscript received February 16, 1981
Revised copy received June 22, 1981

ABSTRACT

The region between 86F1,2 and 87B15 on chromosome 3 of *Drosophila melanogaster*, which contains about 27 polytene chromosome bands including the 87A7 heat-shock locus, has been screened for EMS-induced visible and lethal mutations. We have recovered 268 lethal mutations that fall into 25 complementation groups. Cytogenetic localization of the complementation groups by deficiency mapping is consistent with the notion that each band encodes a single genetic function. We have also screened for mutations at the 87A7 heat shock locus, using a chromosome that has only one copy of the gene encoding the 70,000 dalton heat-shock protein (hsp70). No lethal or visible mutations at 87A7 were identified from 10,719 mutagenized chromosomes, and no female-sterile mutations at 87A7 were recovered from the 1,520 chromosomes whose progeny were tested for female fertility. We found no evidence that a functional hsp70 gene is required for development under laboratory conditions.

IN recent years, considerable effort has been made towards understanding the organization and regulation of eukaryotic genes. One approach has involved the analysis of total nuclear and cytoplasmic transcripts during development (DAVIDSON 1976). This has drawn attention to the relatively small fraction of the eukaryotic genome that is ultimately expressed as cytoplasmic mRNA. A second approach involves the detailed analysis of specific genes. Through studies of the latter type, the interruption of protein-coding sequences by intervening DNA sequences not present in mature mRNA has been demonstrated (ABELSON 1979).

In *Drosophila melanogaster*, the availability of sophisticated genetic techniques allows the adoption of both approaches, with the added advantage that the polytene larval salivary gland chromosomes allow the accurate cytogenetic localization of mutations and purified DNA sequences. Detailed genetic and cytological studies of *D. melanogaster* have suggested that, in general, each band of the polytene chromosomes of larval salivary glands encodes a single genetic unit, and thus the total Drosophila genome codes for about 5,000 genes. This is about one-

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thirtieth of the potential coding capacity of its 1.6×10^8 base pairs (reviewed by LEFEVRE 1974).

A favorable system for studying gene organization in Drosophila is the set of loci that are induced by a brief heat shock (reviewed by ASHBURNER and BONNER 1979). Brief exposure to 37° causes the "puffing" of about nine loci and subsequent synthesis of a specific set of about 8 heat-induced proteins. Previous transcription and translation are repressed by the heat shock, simplifying biochemical characterization of the loci and allowing detailed analysis of their organization and regulation. Two loci in particular, those at 87A and 87C on the right arm of chromosome 3, have been extensively studied. Cloned DNA fragments have been recovered from these sites and have been used to show that both of these loci encode a 70,000 d heat-induced protein (hsp70) (SCHEDL *et al.* 1978; LIVAK *et al.* 1978; ARTAVANIS-TSAKONAS *et al.* 1979; CRAIG, MCCARTHY and WADSWORTH 1979). Parallel genetic experiments have confirmed that there are two hsp70 loci and have mapped them to the single bands 87A7 and 87C1, which are the sites of the heat-induced puffs (ISH-HOROWICZ *et al.* 1979a). These studies have been extended to show that the hsp70-coding sequences are reiterated at the two loci. The total number of hsp70 copies varies from 5 to about 7 according to genotype, two copies being found at 87A7 and 3 to 5 copies at 87C1 (ISH-HOROWICZ *et al.* 1979b; MIRAULT *et al.* 1979; HOLMGREN *et al.* 1979).

In an earlier study, we unsuccessfully attempted to isolate mutations at the 87C1 locus (GAUSZ *et al.* 1979). As the hsp70 loci are duplicated, it is not surprising that no such mutations were recovered; indeed the 87C1 locus in Drosophila appears to be dispensible and can be deleted without greatly affecting viability or fertility (GAUSZ *et al.* 1979; ISH-HOROWICZ and PINCHIN 1980). We have extended our study to cover the region between 86F1 and 87D3,4, covering both hsp70 loci. In part of this work, we have made use of a chromosome variant that has only one functional hsp70 gene (UDVARDY *et al.*, in press), and we present experiments attempting to recover EMS-induced mutations in this gene. From 10,719 mutagenized chromosomes, we recovered 189 lethal mutations, but none localized to the 87A7 heat-shock locus. An additional 9,691 mutagenized chromosomes, which had both hsp70 loci, were also tested without revealing mutations at either 87A7 or 87C1. The 106 lethal mutations recovered in these experiments were assigned to complementation groups, which were mapped onto the polytene chromosomes using a collection of deficiencies. The results are consistent with each chromosome band encoding a single genetic function.

MATERIALS AND METHODS

Drosophila cultures and stocks: Drosophila cultures were maintained on a yeast, cornmeal, sucrose and agar medium. The mutant and balancer stocks used are described in LINDSLEY and GRELL (1968) or are listed in Table 1.

Isolation of recessive mutations within the 86F1,2 to 87D3,4 region of chromosome 3: *Oregon-R-369* males were fed 0.025 M ethyl methansulphonate (EMS) according to the method of LEWIS and BACHER (1968) and mated *en masse* to *CxD/TM3, Sb Ser* virgin females. Mutations were isolated over $Df(3R)kar^{D3}$, as described in Figure 1A. Similar schemes were used to isolate mu-

TABLE 1

List of deficiencies and breakpoints used to divide the region between 86F1,2 and 87D3,4

Deficiency	Breakpoints		Source
	proximal	distal	
Df(3R)T-45	86E	87B5–6	ISH-HOROWICZ
Df(3R)kar^{D3}	86E16–18	87D3,4	RITOSSA
Df(3R)T-10	86F1,2^{x}	87C5–7	ISH-HOROWICZ
Df(3R)E-079	86F1,2	87B9	GAUSZ
Df(3R)T-63	86F1,2^{x}	87A4,5–7	ISH-HOROWICZ
Df(3R)T-47	86F1,2^{x}	87A9	ISH-HOROWICZ
Df(3R)T-61	86F1,2^{x}	87A9	ISH-HOROWICZ
Df(3R)T-07	86F1,2^{x}	86F4–7	ISH-HOROWICZ
Df(3R)T-55	86F1,2^{x}	87A4,5–7	ISH-HOROWICZ
Df(3R)T-41	86F1,2	87C1–2,3	ISH-HOROWICZ
Df(3R)E-229	86F6–7	87B1–2	GAUSZ
Df(3R)kar^{H5}	87A1,2	87D5–7	HENIKOFF
Df(3R)kar^{1W}	87A6–7	87D13–14	ISH-HOROWICZ
Df(3R)kar^{D1}	87A7	87D3,4	RITOSSA
Df(3R)kar^{Sz-15}	87B1–2	87E1–2	GAUSZ
Df(3R)kar^{Sz-12}	87B1–2	87C8–9	GAUSZ
Df(3R)kar^{H1}	87B1	87D12–E1	HENIKOFF
Df(3R)E-307	87B2–4	87D1,2	GAUSZ
Df(3R)kar^{3Q}	87B2–4	87D1,2	ISH-HOROWICZ
Df(3R)kar^{H10}	87B4	87D7–8	HENIKOFF
Df(3R)kar^{H11}	87B5–6	87F1–2	HENIKOFF
Df(3R)ry^{615}	87B11–13	87E8–11	GELBART
Df(3R)kar^{3J}	87B15–C1	87C9–D1,2	ISH-HOROWICZ
Df(3R)ry^{75}	87D1,2	87D14–E1	GELBART

All deficiencies labeled by x carry a residual part of the TE28 transposing element that was used in their isolation. Deficiencies that have identical breakpoints were checked in respect to their genetic lengths and not used for further analysis if they were genetically the same. Where two adjacent bands are indicated, the break lies between them; when only one band is mentioned, the breakpoint is within the band. Otherwise, the outermost band is definitely present; the innermost band is definitely absent.

tations over *Df(3R)T-10* and *Df(3R)E-229*. Experiments 1 and 2 also yielded mutations outside the 86F1,2–87B15 region. Those from 86E16–18 to 86F1,2 were not analyzed further.

The fourth and fifth experiments to recover mutations between 87F1,2 and 87C1 used the *SL-2* chromosome of UDVARDY *et al.*, (in press), which retains only a single functional hsp70 gene derived from a partially deleted 87A7 locus in a *mwh e* stock. Figure 1B describes the mating scheme to identify mutations over *Df(3R)T-41*. In Experiment 5, we also looked for the induction of temperature-sensitive lethal mutations: In 2,125 cases, the F_2 flies were transferred to a fresh tube and incubated for 4 days at 29°. The parents were discarded and the progeny allowed to develop at 25°. Absence of *SL-2/Df(3R)T-41* progeny flies indicated a putative temperature-sensitive lethal. All the treated chromosomes in the *Df(3R)T-10* experiment and 1,520 of the *SL-2* chromosomes in Experiment 5 were checked for mutations affecting female fertility. For this, +*/*Df(3R)T-10* or *SL-2* */*Df(3R)T-41* females were placed with sibling males in fresh vials and the presence of larvae was checked after 4 or 5 days.

We have distinguished the mutations according to the experiment in which they were recovered; the allele designation of mutations recovered in Experiment 1 are prefixed "*e*", Experiment 2 alleles are prefixed "*nf*"; alleles from Experiment 3 are prefixed "*a*", Experiment 4 alleles are prefixed "*hs*", and Experiment 5 alleles are prefixed "*b*". From Experiment 5, only

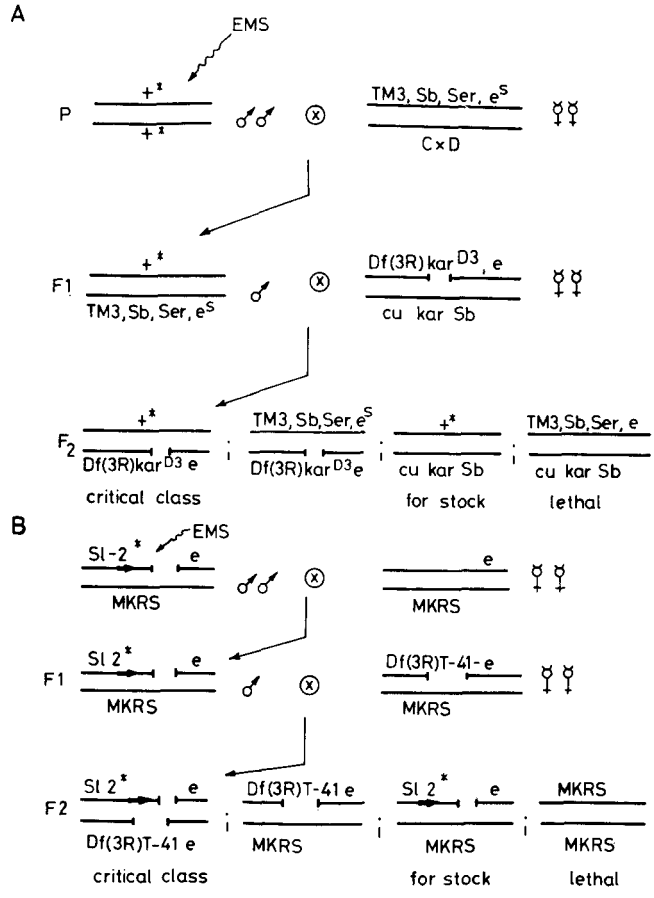


FIGURE 1.—(A) Isolation of mutations over *Df(3R)kar*^{D3}: +/*kar*^{D3} flies were checked for morpholgical abnormalities; absence of the class indicates an induced lethal mutation. Some +*/*Df* females were tested for fertility, as described in MATERIALS AND METHODS. (B) Isolation of mutations over *Df(3R)T41*: *SL-2*/T41* flies were checked for morphological abnormalities. (For a detailed explanation of the *SL-2* chromosome, see MATERIALS AND METHODS.)

the 32 mutations that localized between 86F6,7 and 87B1,2 (*i.e.*, within *Df(3R)E-229*) were analyzed further. Three X-ray-induced mutations, *l(3)ck5*^{t19}, *l(3)ck6*^{t07} and *l(3)ck11*^{r02}, were also included as they map within the region examined.

Deletion mapping of the mutations and assignment to complementation groups: The mutant stocks were assigned to one of 21 subregions by crossing to a series of deficiencies with breakpoints in the region (Table 1; Figure 2). Only the 14 subregions between 86F1,2 and 87B15 are described here, as the others have been presented in a previous publication (GAUSZ *et al.* 1979).

In the first experiment, all mutations within a subregion were assigned to complementation groups by crossing 1 or 2 males to 3 virgin females in three replicate crosses. Crosses giving inconsistent results or inadequate progeny were repeated. Not all alleles of *l(3)ck1* were tested for allelism with each other, as too many mutations were recovered. The probable explanation is that the original Ore-R-369 stock contained a segregating *ck1* allele. In subsequent experiments, allelism was tested by crossing to two of the alleles recovered in Experiment 1.

Lethal phase determination: Representative alleles (showing complete lethality with *Df(3R)T-10*) were selected from each complementation group for the determination of lethal phases.

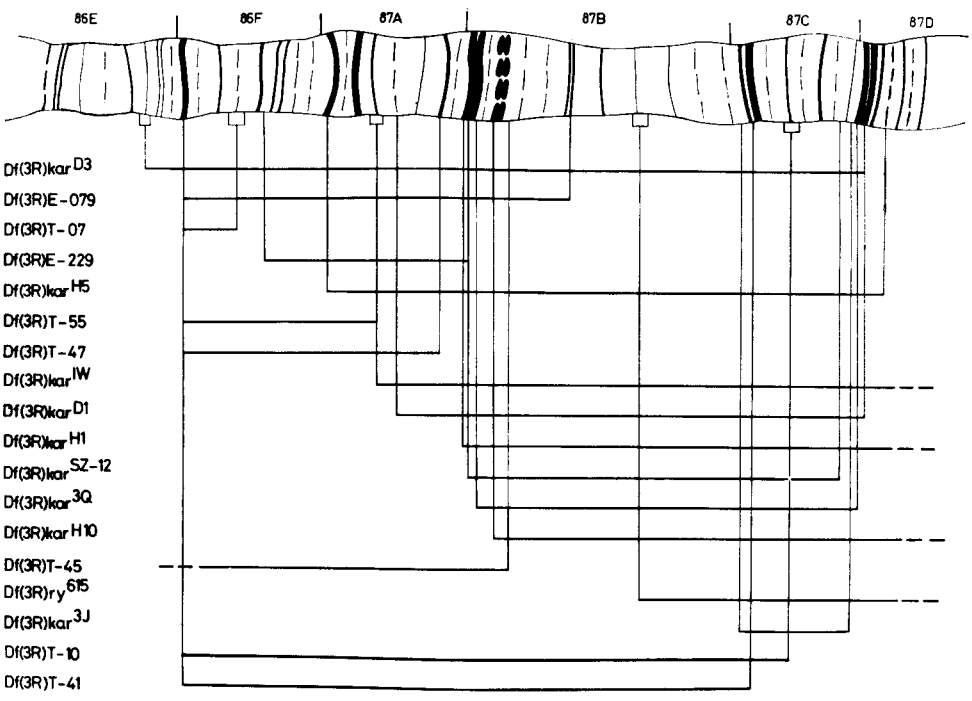


FIGURE 2.—The cytological extent of the deficiencies.

Mutant males were mated to *Df(3R)T-10* virgin females and, after 3 days, eggs were collected at 2 hr intervals on small petri dishes. After 24–28 h at 25°, hatched larvae were counted to estimate egg viability. To determine larval and pupal lethality, 100 eggs were placed in a vial containing standard medium. Adults were counted after eclosion and the medium washed out to count dead larvae and pupae. Ore-R-369/*TM3* males were used in control crosses.

Cytological analysis: Salivary gland preparations were made as described by HOLDEN and ASHBURNER (1978). The proximal and distal breakpoints of all deficiencies were checked, as were preparations of chromosomes from both non-heat-shocked and heat-shocked (37° for 20 min) larvae. A detailed study was made of the banding pattern between 86F1,2 and 87D3,4 for the Ore-R-369 chromosome in which many deletions were induced, and the pattern compared with that described by BRIDGES (1941). We also ranked the band thickness on an arbitrary scale from 1 (thinnest) to 10 (thickest) (Table 2).

RESULTS

Cytological description of the region studied: According to BRIDGES (1941), the region between 86F1 and 87D3,4 contains about 47 bands. Our cytological analysis of Oregon-R-369 chromosomes shows most, but not all, of the bands drawn by BRIDGES. The following bands appear as singlets, rather than doublets: 86F1,2; 87A1,2, 87A4,5; 87C2,3 and 87D1,2. To avoid confusion in band nomenclature, we retain BRIDGES's numbering, but separate the numerals with commas rather than dashes. We have retained BRIDGES's notation for the doublets that we can resolve: *e.g.*, 87B1–2, 87B4–5, 87B8–8 and (probably) 86F8–9. In some of our preparations, 87A9 appears as a doublet. Finally, although we cannot ex-

TABLE 2

Ranking of the bands with respect to their relative thickness

Thickness value in arbitrary units	Bands included in category
1	86F5; 86F10; 87A10; 87B6; 87B7; 87B10; 87B12; 87B14
2	86F4; 87C6; 87C8
3	87A7; 87C8
4	87B8; 87B9; 87B13
5	87A6; 87B15; 87C9
6	86F8; 86F9; 87B8; 87B9; 87B11
7	86F6,7; 87A9; 87C1; 87C5
8	87B4; 87B5; 87C7
9	86F1,2; 87A1,2; 87A4,5; 87B1; 87B2
10	87C2,3; 87D1,2; 87D3,4

Bands not visualized are not ranked.

clude their presence, we have been unable to see the following bands described by BRIDGES: 86F3, 86F11, 87A8 and 87B3.

Analysis of the region is complicated by the presence of two small puffs during the late third larval instar. These are found between 87B5 and B8 and between 87B11 and 87C1. These developmental puffs make visualization of bands 87B12 and 87B14 difficult. We have confirmed the observation of an additional heat-induced puff between 86F3 and 86F6 (ZHIMULEV and GRAFODATSKAYA 1974). This puff is more pronounced during recovery from anoxia, as it appears earlier than the 87A and 87C puffs (H. GYURKOVICS, unpublished observation).

Table 1 lists the breakpoints of the deficiencies used in this study, and Figure 2 indicates their cytological extents. Three of the deficiencies, *Df(3R)E-079*, *Df(3R)E-229* and *Df(3R)E-307*, were induced in Experiment 1 by EMS, a frequency of 1.9×10^{-4}.

Isolation of mutations within 86F1 and 87D3,4: Table 3 gives the yield of mutations within the region of interest for the four experiments. The mutagenized chromosomes in the first three experiments contained both 87A7 and 87C1 heat-shock loci. Experiment 1, using *Df(3R)kar*^{D3}, detected mutations between 86F1 and 87D3,4; Experiment 2, with *Df(3R)T-10*, detected mutations between 86F1 and 87C6; and Experiment 3, with *Df(3R)E-229*, tested for mutations between 86F7 and 87B1. A total of 9,691 chromosomes were tested and 106 mutations recovered.

Experiments 4 and 5 differ from the other experiments in using a chromosome containing only a single functional hsp70 gene. The 87A7 locus of *mwh e* has suffered a partial deletion that inactivates one of its two hsp70 genes (UDVARDY *et al.*, in press). The *SL-2* chromosome used for this experiment combines this partially deleted 87A7 locus with *Df(3R) kar*^{3J}. Thus, *SL-2/Df (3R)T-41* flies totally lack 87C1 and retain only the 87A7 locus of *mwh e* with its single active hsp70 genes (Figure 1). We screened a total of 10,719 EMS-treated *SL-2* chromosomes for lethal and visible mutations; 2,125 chromosomes from Experi-

TABLE 3

The number of isolated mutant alleles and mutation frequencies in the different experiments

Expt. no.	Deletion tested	No. of chromosomes tested	No. of alleles	No. of compl. groups covered	Mutation frequencies per compl. group
1	*Df(3R)kar^{D3}*	8303	72(175)	25	3.5×10^{-4}
2	*Df(3R)T-10*	876	24	25	1.1×10^{-3}
3	*Df(3R)E-229*	512	5	8	1.2×10^{-3}
4	*Df(3R)T-41*	5724	33	25	2.3×10^{-4}
5	*Df(3R)T-41*	4995	157	(25)	1.3×10^{-3}
	Df(3R)kar^{3J}	5726	25	5	8.7×10^{-4}

The number in brackets for Experiment 1 includes all the *l(3)ck1* alleles recovered. They are not included in the calculations for reasons explained in the text. Mutations mapping within the 87C1–9 and 87C1–5 regions recovered in Experiments 1 and 2, respectively, are not included in this analysis. The number of complementation groups in Experiment 5 was not independently determined. The bottom line gives the yields from a previous experiment (GAUSZ *et al.* 1979) for comparison.

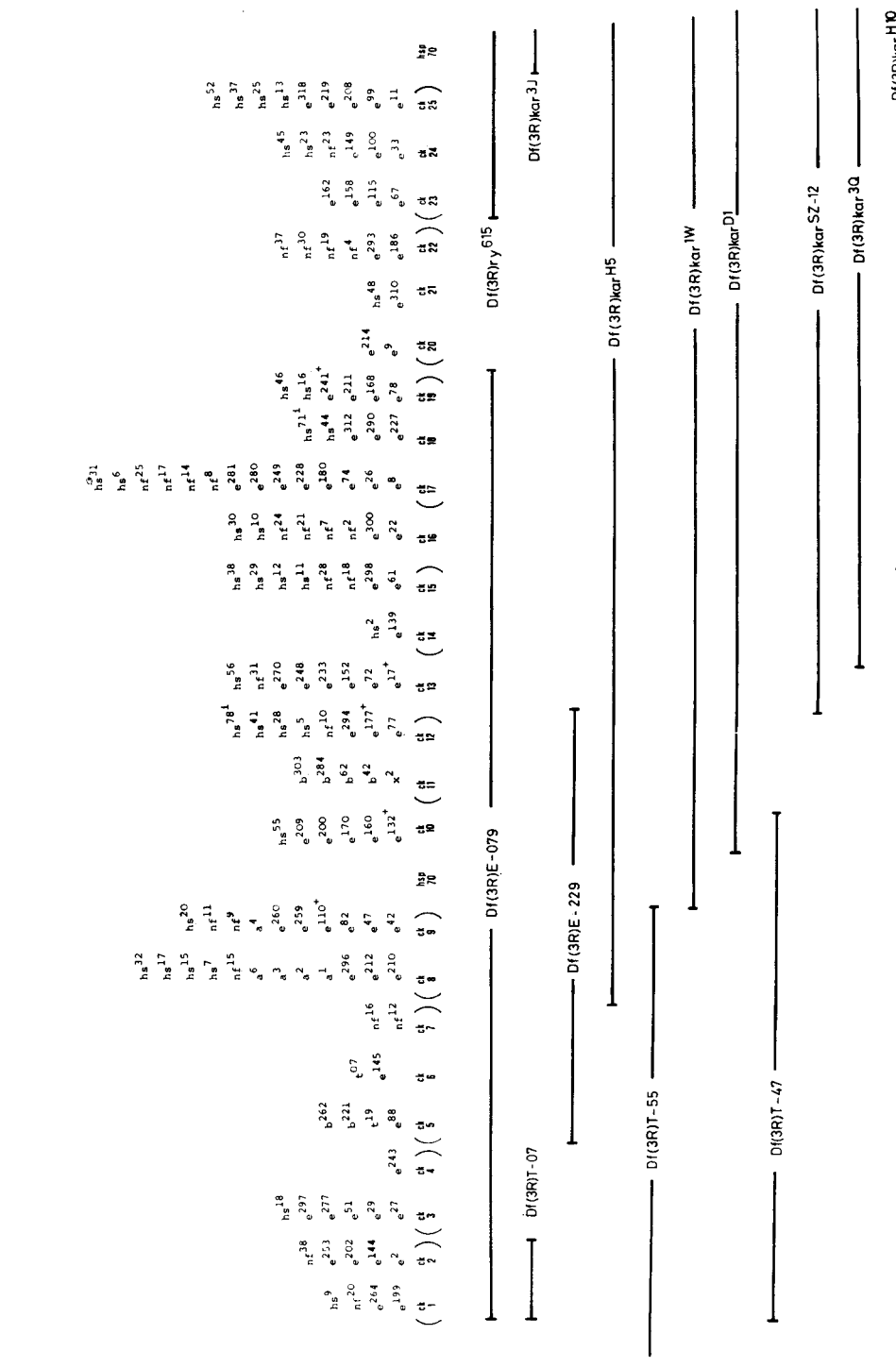
ment 5 were tested for temperature-sensitive lethal mutations, and 1,520 chromosomes from Experiment 4 were further tested for female sterility.

Deletion mapping and mutant characterization: The mutations were first tested against a set of overlapping deficiencies (Table 1; Figure 2). Initial characterization was done with *Df(3R)T-10*, *Df(3R)kar^{1W}*, *Df(3R)kar^{3Q}*, *Df(3R)ry^{615} and DF(3R)kar^{3J}*, followed by finer localization with appropriate remaining deficiencies. In this manner, all the mutations were localized to one of 21 subregions. Mutations within a subregion were crossed *inter se* and allotted to complementation groups accordingly. For reasons discussed below, not all mutations in *l(3)ck1* were crossed to each other. Figure 3 indicates the 25 complementation groups and their cytogenetic localization. The approximate lethal phases of the complementation groups are presented in Table 4. Rather than repeat all the data of Figure 3 and Table 4, we shall comment only on points of interest.

In the first experiment, *l(1)ck1* alleles appeared at a frequency one order of magnitude higher than alleles at other loci. In subsequent experiments, the number of alleles was not proportionally higher, so that we presume that the Ore-R stock in Experiment 1 carried a *ck1* allele. In Experiment 1, *l(3)ck1* and *l(3)ck2* alleles were distinguished by crossing to representatives alleles, whereafter two typical *l(3)ck1* alleles were selected for further study.

We found intragenic complementation within *ck1*. *ck1^{e70}/ck1^{e143}* flies have reduced viability, and the few adults that emerge are very weak, with poor cuticular pigmentation and abnormal wings. Some *l(3)ck13* alleles also show intragenic complementation.

Two mutations, *l(3)ck5^{t19}* and *l(3)ck6^{t07}* were induced by X irradiating the transposing element TE28 (ISING and RAMEL 1976). *ck6^{t07}* was co-induced with the deficiency *Df(3R)T-07*. The other X-ray-induced mutation included in this study, *l(3)ck11^{X02}*, is a late third larval instar lethal. Mutant larvae fail to pupariate and persist as larvae for many days. Their medial and lateral imaginal


Df(3R)T-07
Df(3R)E-079
Df(3R)E-229
Df(3R)T-55
Df(3R)T-47
Df(3R)T-45
Df(3R)kar H5
Df(3R)kar 1W
Df(3R)kar D1
Df(3R)kar SZ-12
Df(3R)kar 3Q
Df(3R)kar H10
Df(3R)ry 615
Df(3R)kar 3J

discs are much reduced in size, and the larvae develop melanotic tumours as they age. The newly induced alleles of this complementation group also contain abnormal imaginal discs. The adjacent complementation group, *l(3)ck12*, is also a late larval lethal, the mutant larvae accumulating crystalline deposits in the anterior portion of the Malphighin tubules. *Df(3R)E-229/Df(3R)kar^{1W}* larvae, which are mutant for both above complementation groups, show both the above phenotypes, suggesting that the alleles examined are amorphic.

DISCUSSION

Nondetection of mutations at the hsp70-coding loci: The 87A7 hsp70 locus is defined cytogenetically by its deletion in *Df(3R)kar^{1W}* and its retention in *Df(3R)kar^{D1}* (ISH-HOROWICZ *et al.* 1979a; Figure 2). No lethal, visible, temperature-sensitive or female-sterile mutations mapping within this region were recovered. Our previous inability to recover mutations at the 87C1 heat-induced locus can be ascribed to the duplication of hsp70 loci and hsp70 genes, especially in view of the viability and fertility of flies lacking the locus (GAUSZ *et al.* 1979). The lack of mutations at 87A7 in the first three experiments of this paper can be similarly explained, as there is evidence that both hsp70 loci are activated by heat shock (LIVAK *et al.* 1978; ARTAVANIS-TSAKONAS *et al.* 1979; ISH-HOROWICZ *et al.* 1979a). However, Experiments 4 and 5 used a chromosome with only a single hsp70 gene (UDVARDY *et al.*, in press); yet, no mutations at 87A7 were found in 10,719 chromosomes. The frequency of mutation per complementation group was 2.2×10^{-4} in Experiment 4 and 1.3×10^{-3} in Experiment 5 (Table 3). The probability of not detecting a lethal hsp70 mutation was 0.28 in Experiment 4 and 0.015 in Experiment 5.The combined probability of not detecting a lethal hsp70 mutation by chance is 4.4×10^{-4}.

We can consider three explanations for the failure to detect an hsp70 mutation. First, hsp70 may be a dispensible protein in Drosphila, despite the structural gene being present in at least five copies per haploid genome. However, the heat-shock response is not limited to Drosophila, but has been described in yeast, avian cells and in slime moulds (MCALISTER *et al.* 1979); KELLEY and SCHLESINGER 1978; LOOMIS and WHEELER 1980). All induce specific proteins after heat shock, and some include a major 70,000 d protein. The Dictyostelium "hsp" resolves into two peptides on two-dimensional gel electrophoresis one of which co-migrates with Drosophila hsp70 (LOOMIS and WHEELER 1980). Conservation between such widely diverse species suggests that it is an important protein and argues against

FIGURE 3.—Deletion mapping of the complementation groups. The complementation groups between 86F1,2 and 87B15 are indicated relative to the deficiency breakpoints. Complementation groups within brackets are unordered. Individual alleles are listed above the respective loci. Alleles from Experiments 1,2,3,4 and 5 (Table 2) are designated "*e*", "*nf*", "*a*", "*hs*" and "*b*", respectively (the "*b*" alleles of only *ck5* and *ck11* are listed in the figure). Also included are *l(3)ck5^{t19}* and *l(3)ck9^{t07}*, which were induced by irradiating a chromosome containing a TE28 transposing element and *l(3)ck11^{x02}*, an independently isolated X-ray-induced mutation. + marks semi-lethal alleles; i marks temperature-sensitive alleles.

TABLE 4

Lethal phases of the complementation groups

Compl. group	Alleles	Phase of lethality	Phenotype
ck1	e^{199} e^{264}	1st-2nd larval	intragenic complementation
ck2	e^{2}	2nd larval	larvae leave the medium and die
	e^{144}	2nd-3rd larval	
ck3	e^{27}	pupal	
	e^{277}	pupal/adult	adult escapers with abnormal morphology
ck4	e^{243}	egg-larval-pupal	
ck5	e^{88}	1st-2nd larval	
ck6	e^{145}	1st-2nd larval	
ck7	nf^{12} nf^{16}	1st larval	uninflated trachea
ck8	e^{210}	3rd larval	
	e^{296}	3rd larval	late 3rd instar form tanned pseudopupae
ck9	e^{82} e^{260}	egg	
ck10	e^{200} e^{209}	late pupal	formed pupae are longer than normal
ck11	x^{02}	late 3rd larval	imaginal discs strongly reduced in size
ck12	e^{77}	larval-late pupal	abnormal morphology of Malphighian tubes
	e^{177}	late pupal	abnormal morphology of Malphighian tubes
ck13	e^{233} e^{270}	1st-2nd larval	intragenic complementation
ck14	e^{139}	1st-2nd larval	
ck15	e^{61}	1st-2nd larval	
	e^{298}	1st-2nd larval	
ck16	e^{22}	egg-1st larval	
	e^{300}	egg	
ck17	e^{26}	1st-2nd larval	
	e^{180}	1st-3rd larval	
ck18	e^{290} e^{312}	early pupal	young pupae undergo autolysis
ck19	e^{168}	late pupal	fully formed pigmented pharate adults
	e^{211}	2nd-3rd larval	
ck20	e^{9} e^{214}	1st-2nd larval	
ck21	e^{310}	1st-3rd larval	
ck22	e^{186}	2nd-3rd larval	
	e^{293}	1st-2nd larval	
ck23	e^{115}	1st-2nd larval	
	e^{158}	1st-3rd larval	
ck24	e^{33} e^{100}	early pupal	young pupae undergo autolysis
ck25	e^{99} e^{219}	1st-3rd larval	
Ore-R-369		normal adults	

All of the studied alleles were crossed to *Df(3R)T-10* virgins in order to determine lethal phase.

its dispensibility. A second explanation is that we have not yet found the correct conditions for identifying the phenotype of a fly lacking functional hsp70 genes. We have assayed for viability at 25° and 29°, and for fertility at 25°. Perhaps flies lacking hsp70 have a conditional phenotype that is not expressed in our experiment. A final possibility arises from the observations of HOLMGREN *et al.* (1979) that the gene for the 68,000 d heat-shock protein (hsp68) shows considerable homology to the hsp70 gene. If hsp68 can substitute for hsp70, we will not recover hsp70 mutations. We note that hps68 synthesis is enhanced by the deletion of hsp70 genes (ISH-HOROWICZ, HOLDEN and GEHRING 1977; ISH-HOROWICZ *et al.* 1979a).

The genetic organization of the 86F1,2 to 87D3,4 region: Our results on the genetic organization of the 86F to 87C region are in accord with previous suggestions that most bands/interbands of the giant salivary gland chromosome encode single genetic units (JUDD, SHEN and KAUFMAN 1972; LEFEVRE 1974). The large region studied and the large number of mutations recovered permit a more detailed analysis than heretofore. BARRETT (1980) presented an analysis of saturation-mapping experiments, fitting the mutation frequencies to a truncated Poisson distribution and estimating the probable number of undetected lethal-mutable loci. Previous data were either inadequate or inconsistent with a Poisson distribution (BARRETT 1980). We analyzed the combined data on the 86F1,2 to 87B15 region from Experiments 1 and 4 (being comparable in scale and in the efficiency of recovery of mutations, and found that it is consistent with a truncated Poisson distribution of parameter $m = 4.59 \pm 0.46$ (goodness-of-fit $\chi^2_2 = 1.76$; $p > 0.25$). This allows us to estimate the number of loci undetected by chance as $n_0 = 0.23 \pm 0.10$. We already know of two complementation groups missed in Experiments 1 and 4 (*ck7* and *ck11*), but the analysis strongly suggests that there are few, if any, additional lethal loci to be defined between 86F1,2 and 87B15. We, of course, exclude loci that are dispensable for normal development.

Within 86F1,2 and 87D1,2, a region containing about 35 bands, we have found 30 discrete complementation groups (29 lethal and 1 visible). We also know of four additional regions that code for nonessential (or nonmutable) functions: 87A7 and 87C1 both encode hsp70; 87C2,3 codes for function(s) necessary for female fertility (GAUSZ *et al.* 1979); 87D1,2 encodes malic enzyme (VOELKER *et al.* 1981). Thus, the correlation between bands and genetic functions is almost exact. In several cases (GAUSZ *et al.* 1979), we were able to map complementation groups to single bands, supporting the notion of the band as the cytological reflection of a genetic unit. We note that other genes have been attributed to the 87B region: dipeptidase C (VOELKER, personal communication), $tRNA^{Lys}_5$ (HAYASHI *et al.* 1980) and a modifier of position-effect variegation (HENIKOFF 1979), but their relationship to the mutations described herein remains unclear.

A consequence of the gene-band relationship should be the lack of correlation between the number of complementation groups in a region and its DNA content. We have estimated DNA content by ranking band thickness between 86F1,2 and 87D3,4 on an arbitrary scale from 1 to 10: 1 for the thinnest band, such as 87B8;

10 for dense bands, such as 87C2,3 or 87D3,4 (Table 2). The number of complementation groups in each region correlates well with the number of bands, but poorly with the ranked DNA content (Table 5). Nor are the number of alleles recovered related to this measure of DNA content. One might expect that, in general, mutation frequencies should be dependent on the length of target DNA, although modified by the organization of the specific gene and the degree to which the gene product can tolerate mutational changes. This is suggested by the observation that higher molecular weight proteins show higher heterozygosity in Drosophila populations than do smaller ones (KOEHN and EANES 1977; LEIGH BROWN and LANGLEY 1979). The lack of relationship between band size and mutation frequency may be due either to the coding regions being restricted to a small part of the band (SORSA, GREEN and BEERMAN 1973; BEERMAN 1972;

TABLE 5

Pooled data for subregions: DNA content, band number, complementation groups and allele totals

Regions	No. of bands	Total ranked band thickness	No. complementation groups	Total no. alleles
86F1,2;F4	3	10	2	7(104)
86F5;F6,7	2	8	2	7
86F8;87A1,2*	3.5	17.5	3	4
87A1,2*,A6	2.5	18.5	2	17
87A7	1	3	—	—
A9*	0.5	3.5	1	6
87A9*,B1	2.5	13.5	2	8
87B2	1	9	1	8
87B4*	0.5	4	2	10
87B4*,B5	1.5	12	1	8
87B6;B9*	3.5	8	3	25
87B9*,B12	3.5	10	3	10
87B13;B15	3	10	3	19
87C1	1	7	—	—
87C2,3	1	10	—	—
87C4,5	1	8	1	13
87C5	1	2	1	6
87C7	1	8	1	5
87C8	1	2	1	13
87C9	1	5	1	15
87D1,2	1	10	—	—

Data from Experiment 3 are not included, as they covered only part of the 86F1,2–87B15 region. The *ck5* and *ck11* alleles from Experiment 5 are also excluded, as the other mutations recovered in this screen were not assigned to individual complementation groups. The data for the 87C1–9 region are taken from GAUSZ *et al.* (1979) in which 14,029 chromosomes were tested (compared to a total of 14,903 in Experiments 1,2 and 4).

* Marks bands broken by a deficiency; in such cases, its ranking is shared between the adjacent subregions.

The total number of alleles in parenthesis between 86F1,2 and 86F4 includes the *l(3)ck1* alleles believed to originate from the mutagenized chromosomes. Any EMS-induced alleles will be included in this number, so that their exclusion leads to a slight underestimate of the number of induced alleles in this region.

VOELKER *et al.* 1981) or to the interruption of protein-coding regions by intervening DNA sequences.

Lethal phenotypes in the region: There is no obvious similarity between the lethal phases of loci flanking the 87A7 and 87C1 loci; 19 of the 29 lethal loci between 86F1,2 and 87C9 are larval lethals. Three complementation groups show embryonic lethality, six are pupal lethals and one is polyphasic. It has previously been suggested that many developmentally important zygotic genes are subject to a maternal effect that can provide wild-type product to prolong development (GARCIA-BELLIDO and MOSCOSO DEL PRADO 1979). This may explain the preponderance of larval over embryonic lethals.

Future work: We wished to induce mutations at the 87A7 hsp70 locus to permit genetic analysis of its expression and function. Our inability to detect such mutations indicates that we must find conditions in which hsp70 production is necessary for the fly. Evidence suggests that synthesis of heat-shock proteins may be necessary for repression of their own induction and resumption of normal transcription following the return to lower temperatures. (ARRIGO 1980; VELAZQUEZ, DIDOMENICO and LINDQUIST 1980). Thus, flies lacking active hsp70 may be particularly susceptible to heat shock, and experiments to test this are underway. In addition, we hope to isolate cloned DNA fragments that extend into the regions adjacent to 87A7 and 87C1, allowing biochemical characterization of the adjacent loci.

We should like to thank SHEENA M. PINCHIN for the isolation and initial characterization of the *T*-series deficiencies.

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