MUTATIONAL ANALYSIS OF THE REGION SURROUNDING THE 93D HEAT SHOCK LOCUS OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

The region containing subdivisions 93C, 93D and 93E on chromosome *3* of *Drosophila melanogaster* has been screened for visible and lethal mutations. Treatment with three mutagens, γ irradiation, ethyl methanesulfonate and diepoxybutane, has produced mutations that fall into 20 complementation groups, including the previously identified ebony locus. No point mutations affecting the heat shock locus in 93D were detected; however, a pair of deficiencies that overlap in the region of this locus was isolated. Flies heterozygous in *trans* for this pair of deficiencies are capable of producing all of the major heat shock puffs (except 93D) and the major heat shock proteins. In addition, these flies show recovery of normal protein synthesis following a heat shock.

THE heat shock response of *Drosophila melanogaster* involves the coordinate activation of a small set of genes. When cells are shifted from 25° to 37°, the synthesis of a small set of proteins and RNAs is enhanced, whereas synthesis of most previously synthesized proteins and RNAs is curtailed (TISSIERES, MITCHELL and TRACY 1974, SPRADLING, PENMAN and PARDUE 1975). In polytene tissues, puffs are induced on the polytene chromosomes at the loci of the transcription of many of the new RNAs.

The heat shock locus at 93D differs from the other major loci in many respects. This region forms one of the largest heat shock puffs and is a major site of active transcription after a shift to 37° (BONNER and PARDUE 1976). In spite of the high RNA production, 93D does not appear to code for any of the major heat shock proteins, which have been mapped to the other large heat shock puffs (see review by ASHBURNER and BONNER 1979). Although RNAs complementary to the other major heat shock loci are predominately cytoplasmic, much of the heat shock RNA complementary to 93D is in the nucleus. Unlike the other heat shock RNAs, the 93D heat shock RNA is predominantly polyA$^-$ in the cytoplasm, whereas the nuclear 93D heat shock RNA is both polyA$^+$ and polyA$^-$ (LENGYEL *et al.* 1980).

Although a puff at 93D is always induced during a heat shock response, the

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extent of activation at 93D can be varied with respect to the activity of the other heat shock loci by environmental conditions (BONNER and PARDUE 1976). In addition, a puff at 93D can be induced independently of the other heat shock puffs by benzamide or by incubating salivary glands in homogenates of heat-shocked salivary glands (LAKHOTIA and MUKERJEE 1980). In every Drosophila species studied, the heat shock loci include one major puff site that shares these unusual inducibility characteristics of the 93D locus. In other Drosophila species the locus is also inducible with vitamin B_6, although *D. melanogaster* does not respond to vitamin B_6. The 93D-equivalent puff in *D. hydei* also encodes RNA that is found predominantly in the nucleus and appears to be poly-A^+ (LUBSEN *et al.* 1978).

Although the unusual features of the 93D-equivalent heat shock puff show strong evolutionary conservation, the DNA sequences encoding the RNA appear to evolve rapidly. There is no detectable cross-hybridization of these sequences between *D. melanogaster*, *D. virilis* or *D. hydei* (PETERS, LUBSEN and SANDERMEIJER 1980). In contrast, sequences encoding the major heat shock proteins show strong cross-hybridization between these species.

One approach to deciphering this unique locus's role in the heat shock response and in the general physiology of the fly is to analyze the effect of mutations at 93D. Mutations affecting the 93D heat shock locus can be identified by their failure to complement deficiencies that delete the heat shock locus. Recently, we have obtained a series of deficiencies in the 93C–E region and localized the heat shock locus relative to these deficiencies by cytological criteria (MOHLER and PARDUE 1982). The heat shock locus was localized by three criteria: whether the deleted chromosome was capable of puffing in 93D following a temperature shift, whether the deleted chromosome was capable of transcription in the 93D region following a temperature shift and whether the deleted chromosome contained sequences in the 93D region complementary to heat shock RNA as determined by *in situ* hybridization. These three criteria gave essentially the same results and localized the 93D heat shock locus to the cytological region 93D6–7.

The deficiencies that, by cytological criteria, delete the heat shock locus have now been used to screen for mutations in the region of this locus. This report describes the mutations that fail to complement these deficiencies as well as the physiological effects of deficiencies for the 93D heat shock locus.

MATERIALS AND METHODS

Culture conditions and genetic strains: All Drosophila stocks and crosses were grown on a standard cornmeal-molasses medium. Stocks were maintained at 21°, and crosses (unless otherwise indicated) were raised at 25°. Except for *TM6B*, a third chromosomal balancer obtained from L. CRAYMER, all of the genetic variants used in this study are described by LINDSLEY and GRELL (1968). The deficiencies used in these studies are described either by MOHLER and PARDUE (1982), SCALENGE and RITTOSSA (1977) or HENIKOFF (1980).

Generation of 93D region mutations: Figure 1 shows the generalized cross-scheme used to screen for mutations in the 93D region. Males mutagenized with either γ-irradiation, ethylmethane sulfonate (EMS) or diepoxybutane (DEB) were mated *en masse* for 4 days to females bearing the *TM3* balancer (either $Df(3R)ws^{28}/TM3$ or $Df(3R)e^{Gp4}/TM3$). The females were transferred to new food for another 4 days and then discarded. Progeny males bearing a mutagenized third chromosome

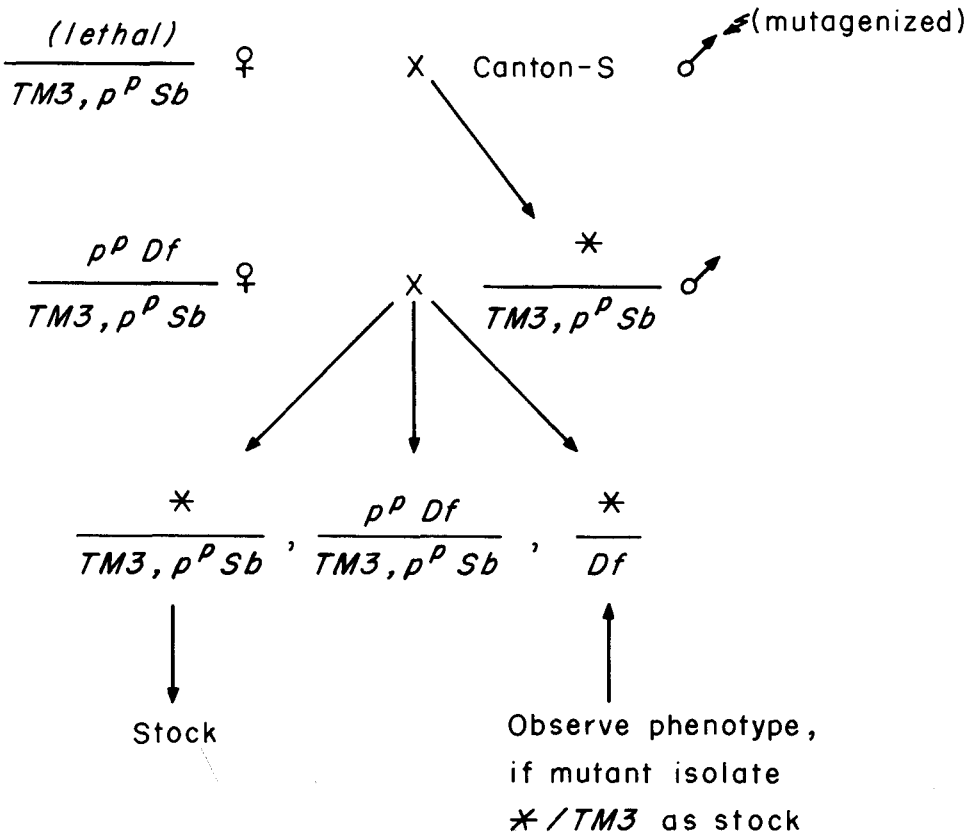

FIGURE 1.—Generalized cross-scheme used to isolate mutations in the 93D region. Canton-S males were mutagenized and mated to *TM3*-bearing females. Progeny males heterozygous for a mutagenized third chromosome and *TM3* were mated to a deficiency for the 93D region. Flies heterozygous for the mutagenized chromosome (*) and the deficiency were examined in the next generation. If these were abnormal or absent, stocks of the flies heterozygous for the mutagenized chromosome and *TM3* were retained.

and the *TM3* balancer were mated individually to females bearing a 93D deficiency and *TM3* (either p^p *Df(3R)*e^{H4}/*TM3* or p^p *Df(3R)*e^{Gp4}/*TM3*). In crosses in which the progeny heterozygous for the mutagenized third chromosome and the deficiency were either missing or visibly mutant, sibs heterozygous for the mutagenized third chromosome and *TM3* were retained as a stock.

All alleles generated in this study were named according to the source of the mutagenized chromosomes and the methods of mutagenesis. The first letter of the allele name identifies the mutagen used: *E* designates EMS, *D* designates DEB, *G* designates γ-irradiation. The second letter of the allele name identifies the source of the mutagenized chromosome: *p* designates the p^p chromosome, *C* designates the Canton-S chromosome. The alleles were numbered in the order of isolation. Complementation groups were named *er* (ebony region) and numbered consecutively as alleles in these groups were isolated. Mutations were considered lethals if progeny heterozygous for the mutagenized chromosome and a 93D deficiency had less than 1% viability, semilethals if the mutant/deficiency heterozygotes had less than 20% viability and visible mutations if more than half the mutant/deficiency heterozygotes expressed the visible phenotype.

Mutations *GC24* to *GC33*, *EC24* to *EC44* and *DC1* to *DC4* were isolated in screens involving a mild heat shock. Seven days after the males bearing a mutagenized third chromosome and *TM3* were mated to deficiency-bearing females, the vials were shifted to 34° for 2 hr and then returned to 25° for the remainder of the experiment.

γ-Irradiation mutagenesis: Mutants *GC1* to *GC23* were generated in chromosomes from a Canton-S stock by exposure to 4000 r from a Cs^{137} γ-radiation source (130 r/min) and were identified by their failure to complement $Df(3R)e^{Gp4}$ in the mutagenesis screen. The ebony region of each mutant chromosome was transferred into the multiply marked rucuca chromosome for complementation tests involving these mutations.

Mutants *GC24* to *GC33* were generated by exposure to 4000 r from a Cs^{137} γ-radiation source. Mutagenized males were from a subline, CS-3, homozygous for a single viable third chromosome from Canton-S. The mutants *GC24* to *GC33* were identified by their inability to complement $Df(3R)e^{H4}$.

EMS-induced mutagenesis: The mutagenesis procedure of LEWIS and BACHER (1968) was followed, with 0.0125 M EMS as suggested by AARON and LEE (1977). Mutants *EC1* to *EC4* were generated in chromosomes from a Canton-S line and were identified by their inability to complement $Df(3R)e^{Gp4}$. Mutants *EC7* to *EC28* and *EC30* to *EC32* were generated in chromosomes from the CS-3 subline of Canton-S and were identified by their inability to complement $Df(3R)e^{Gp4}$. Mutants *EC29* and *EC34* to *EC44* also were generated in *CS-3* chromosomes but were identified by their inability to complement $Df(3R)e^{H4}$.

DEB mutagenesis: Male flies were exposed to DEB for 24 hr in shell vials in which Kimwipes had been inserted and moistened with 0.01 M DEB in 1% sucrose. The males were removed for mating, and all materials were decontaminated by soaking in 1% sulfuric acid for 24–48 hr. Mutants *DC1* to *DC4* were generated in chromosomes from the CS-3 stock and were identified by their inability to complement $Df(3R)e^{H4}$.

Heat shock protein synthesis: Ovaries from $Df(3R)e^{Gp4}/Df(3R)GC14$ females and from Canton-S females were dissected out in Minimal Robb's Medium [(MRM), FRISTROM, LOGAN and MURPHY 1973] and placed individually in 16 μl of MRM in an Eppendorf tube. The Eppendorf tubes were suspended in a 37° water bath for 0.5 hr and then 4 μl of 5 mCi/ml ^{35}S-methionine were added after 0, 1, 2 or 4 hr recovery at 22°. These cultures were labeled at 22° for 0.5 hr. Then, 4 μl of 5× sample buffer were added to each tube to prevent further incorporation. Polypeptides were analyzed by autoradiography after electrophoresis on 10–20% polyacrylamide gradient gels at 16 mA for 5 hr.

Heat shock viability: The relative viability of the *trans*-heterozygote $Df(3R)e^{Gp4}/Df(3R)GC14$ was determined after various heat shock exposures. The cross $Df(3R)e^{Gp4}/TM6B \times Df(3R)GC14/TM6B$ was established in single vials, maintained at 22° and transferred to fresh food after 4 and 8 days. For each cohort, vials containing 1- to 5-day-old larvae were given one of four experimental treatments or were left as untreated controls. Treatments were arranged in a Latin Square design to test for cohort and parental effects. Pupae in these vials were scored for the Tb phenotype (carried on *TM6B*) 5 days later. The total heat shock viability is measured as the proportion of total pupae in the treated vials compared with the total pupae in untreated vials. The relative viability of the $Df(3R)e^{Gp4}/Df(3R)GC14$ *trans*-heterozygote is twice the ratio of Tb^+ to Tb individuals after each treatment.

RESULTS

The 93D region of chromosomes bearing either of the deficiencies, $Df(3R)e^{Gp4}$ or $Df(3R)e^{H4}$, fails to puff and possesses little, if any, transcriptional activity under heat shock conditions. These deficiencies retain no detectable homology to heat shock RNA (MOHLER and PARDUE 1982). $Df(3R)e^{Gp4}$ and $Df(3R)e^{H4}$, therefore, appear to be deficient for most, if not all, of the 93D heat shock locus. These deficiencies were used to screen for mutants in the region of the 93D heat shock locus. Lethal, as well as visible, mutations were selected by their failure to complement $Df(3R)e^{Gp4}$ or $Df(3R)e^{H4}$ either for viability or normal anatomy. Figure 1 shows a generalized cross-scheme used to screen for mutants in this region. Initially, $Df(3R)e^{Gp4}$ was utilized in the mutagenesis screen. However, in the course of this work it became apparent that the distal endpoint of the deletion in $Df(3R)e^{Gp4}$ was very close to the heat

shock locus, whereas the deletion in $Df(3R)e^{H4}$ extended well past either side of the heat shock locus. Therefore, $Df(3R)e^{H4}$ was incorporated into the screen so that it could be assured that the screen involved a complete deletion of the heat shock locus.

A total of 13 γ-irradiation-induced mutations (out of 5286 mutagenized chromosomes screened) and 25 EMS-induced mutations (out of 4281 chromosomes screened) were isolated by the failure to complement $Df(3R)e^{Gp4}$. An additional 10 γ-irradiation-induced mutants (out of 1923 chromosomes screened), 11 EMS-induced mutations (out of 384 chromosomes screened) and four DEB-induced mutations (out of 1199 mutagenized chromosomes) were obtained by the failure to complement $Df(3R)e^{H4}$. The cytology of mutations generated by γ-irradiation with chromosomal aberrations are listed in Table 1. The phenotypes of nonlethal mutations are listed in Table 2. In addition, three irradiation-induced and seven EMS-induced ebony mutations were identified in the first generation of the mutagenesis screen by failure to complement the ebony allele on *TM3*.

These mutations were localized within the 93C–F region by their comple-

TABLE 1

Cytology of γ-induced mutations in the 93D region with chromosome abnormalities

Mutant	Cytology
GC3	*Df(3R)93C;94A*
GC9	*Df(3R)93B;93D*
GC18	*In(3R)81F;93C*
GC21	*In(3R)91A;93C*
GC23	*In(3R)81F;93D*
GC25	*In(3R)93E;94A*

TABLE 2

Phenotypes of nonlethal mutations isolated in the 93D region

Mutant	Locus	Phenotype	
		Homozygous	Over deficiency
GC23	*er9*	Rough eyes	Rough eyes
GC26	*er18*	Lethal	Semilethal
GC30	*er19*	Lethal	Semilethal
GC32	*er12*	Lethal	Semilethal
EC16	*er4*	Lethal	Wings held out
EC19	*er1*	Lethal	Semilethal
EC24	*er2*	Temperature sensitive lethal	Temperature sensitive lethal
EC29	*er18*	Warped wings/female sterile	Warped wings/female sterile
EC35	*er12*	Lethal	Semilethal
EC38	*er11*	Lethal	Semilethal, warped wings
EC39	*er12*	Lethal	Semilethal

Included are the phenotypes when homozygous and when heterozygous with the deficiency with which the mutation was isolated. Phenotypes are described in more detail in the text. (The homozygous phenotype may be due to other mutations on the mutant chromosome.)

mentation behavior with a series of overlapping deficiencies for this region. The breakpoints of the deficiencies used in this study are listed in Table 3. Pairwise complementation tests were conducted on all mutations that failed to complement $Df(3R)e^{Gp4}$. All mutations that failed to complement $Df(3R)e^{H4}$ were also pairwise mated. These tests divided the mutations into 20 complementation groups, including the ebony locus. The resultant complementation map is shown in Figure 2. One mutation, *GC14*, appeared to be a deficiency by genetic criteria (see following data), although the deficiency was not detected cytologically (MOHLER and PARDUE 1982). The mutations that failed to complement $Df(3R)e^{Gp4}$ fell into 12 complementation groups, including the ebony locus. An additional eight complementation groups were identified to the right by complementation with $Df(3R)e^{Gp4}$ but not $Df(3R)e^{H4}$.

The er4, er7 *and* er6 *complementation groups*: These three complementation groups map to the 93C region and are separated from the other complementation groups of this region by five deficiency breakpoints. Approximately half of the mutations isolated by the failure to complement $Df(3R)e^{Gp4}$ fall into these three complementation groups. This region is divided by one deficiency breakpoint. $Df(3R)e^{F4}$ complements all *er4* and *er7* mutations but fails to complement all *er6* mutations. No deficiency was found that separates *er4* from *er7*.

Although most alleles of the *er4* complementation group complement most alleles of the *er7* group, *EC16* is lethal in combination with *EC9* and *EC10* but complements the other *er7* alleles. This is especially noteworthy since *EC16* is a visible mutation (with outspread wings) in combination with $Df(3R)e^{Gp4}$, $Df(3R)e^{GC9}$, $Df(3R)e^{R1}$ and $Df(3R)e^{H5}$ and all of the lethal alleles of *er4*. It is not clear whether this represents negative complementation of the *EC16* and *EC9*, *EC10* mutations or whether this represents a common lethality outside the

TABLE 3

Breakpoints of deficiencies used in this study

Deficiency	Breakpoints	
	Proximal	Distal
$Df(3R)e^{Gp4}$	93B11–13	93D7–9
$Df(3R)e^{GC3}$	93C3–6	94A
$Df(3R)e^{GC9}$	93B11–13	93D9–10
$Df(3R)e^{R1}$	93B3–5	93D2–4
$Df(3R)e^{H4}$	93C3–6	93F6–8
$Df(3R)e^{H5}$	93B11–13	93D4–6
$Df(3R)e^{H6}$	93C3–6	94A
$Df(3R)e^{F1}$	93B8–13	93D7–10
$Df(3R)e^{F2}$	93A6–B1[a]	93D7–10
$Df(3R)e^{F3}$	93B8–13	93E6–11[a]
$Df(3R)e^{F4}$	93C3–6	93F11–14[a]
$Df(3R)e^{D7}$	93C3–6	93F6–8[a]

More complete descriptions of these deficiencies are given by SCALENGE and RITOSSA (1977) and MOHLER and PARDUE (1982).

[a] Breakpoints differ from those of SCALENGE and RITOSSA.

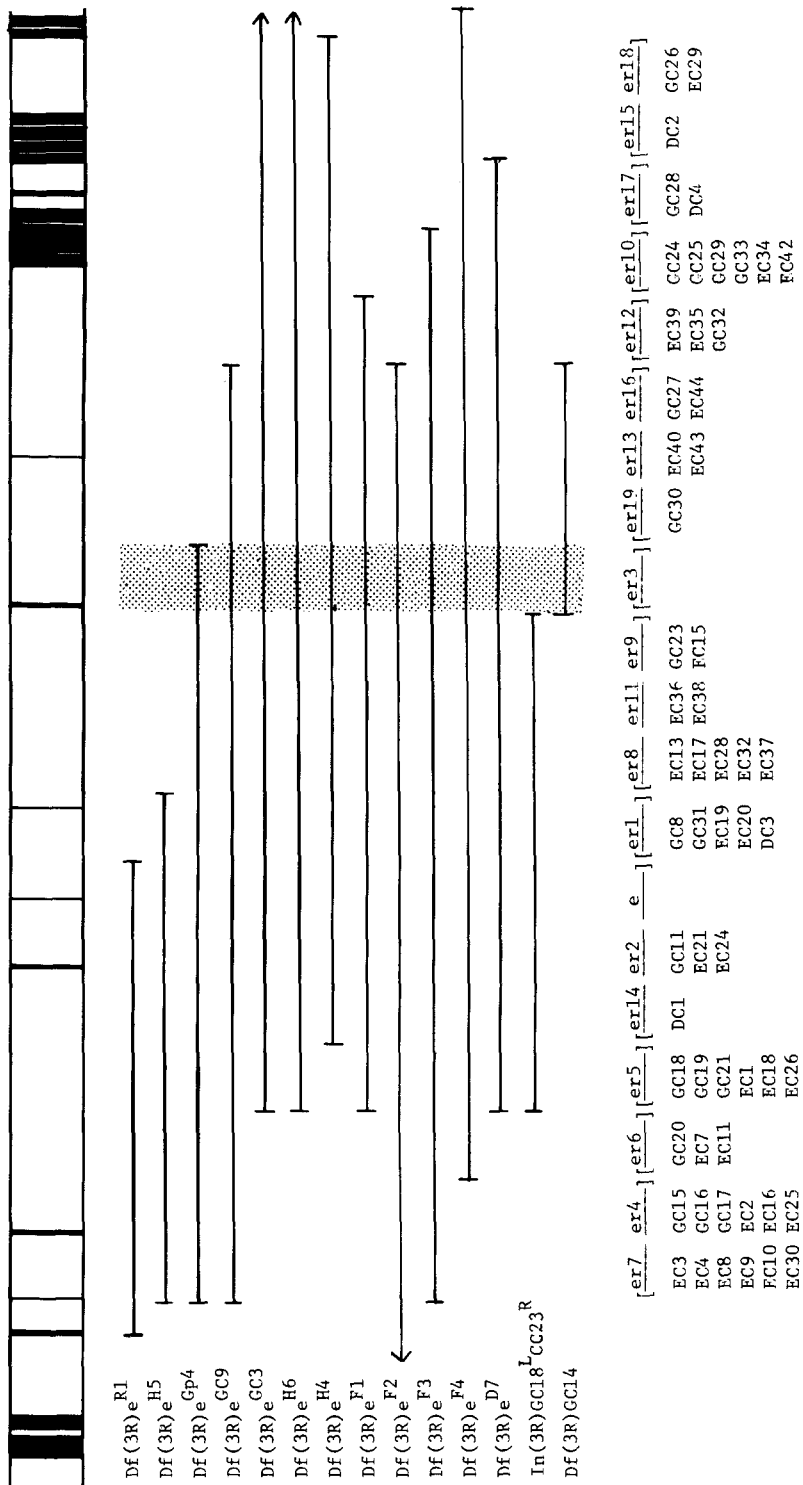

FIGURE 2.—Complementation map of lethals isolated by failure to complement $Df(3R)e^{Gp4}$ or $Df(3R)e^{H4}$. Deficiencies are placed according to their complementation behavior with respect to the point mutations. The polytene chromosome bands have been placed to fit as many deficiencies as possible. An exceptional deficiency is $Df(3R)e^{F1}$, which appears to delete 93C1–2 but complements *er7*, *er4* and *er6* mutations; all other deficiencies that delete 93C1–2 fail to complement *er7*, *er4* and *er6*. The shaded region indicates the location of the 93D heat shock locus as indicated by MOHLER and PARDUE (1982).

93C–D region. However, since *EC9* (but not *EC10*) is lethal over *EC21* (but not the other alleles of *er2*), which are separated by a number of deficiency breakpoints, it may be that this is due to a common lethality outside the region.

The er5, er14, er2 *and ebony complementation groups*: These complementation groups fail to complement most of the ebony deficiencies. They are separated by one deficiency breakpoint: *er5* complements $Df(3R)e^{H4}$, whereas *er14* and *er2* do not. The *er5* complementation group consists of six mutations, two of which are inversions. In addition, four of the 12 known ebony deficiencies have breakpoints within or adjacent to this locus. This complementation group appears to define a hot spot for double-stranded breaks.

The *er14* and *er2* complementation groups are not separated from the ebony locus by any deficiency breakpoint. The *er2* complementation group has three members, *GC11*, *EC21* and *EC24*. *EC24* is a temperature-sensitive allele, which is lethal at 29° and homozygous viable at 25°. One mutation, *DC1*, defines the *er14* complementation group. Recombination mapping placed *er14* approximately 0.2 map unit to the left of ebony. Females heterozygous for *DC1* and *ss e tx* were mated to $Df(3R)e^{Gp4}/TM3$ males, and two deficiency-bearing, e^+ viable progeny were recovered (of 3021 total progeny). Both chromosomes retained the left-flanking *ss* marker and lost the right-flanking *tx* marker.

The er1, er8, er11 *and* er9 *complementation groups*: These four complementation groups all fail to complement $In(3R)GC18^{L}GC23^{R}$ and $Df(3R)e^{Gp4}$, and all complement $Df(3R)e^{R1}$. The *er1* complementation group fails to complement $Df(3R)e^{H5}$, which is complemented by the other three (*er8*, *er11*, *er9*). Approximate recombination data indicate that these four loci form a tightly clustered group. The complementation map places *er1* at the left end of this cluster [it fails to complement $Df(3R)e^{H5}$] and *er9* at the right end of this cluster [$In(3R)GC18^{L}GC23^{R}$, a recombinant chromosome from *In(3R)GC18* (*er5*) and *In(3R)GC23* (*er9*), which deletes the loci between the breakpoints of these inversions, fails to complement alleles of the *er1*, *er8*, *er9* and *er11* loci]. Only one recombinant was isolated between members of *er1* and *er9* (0.05 map unit; females *trans*-heterozygous for *EC20* and *EC15* were mated to $Df(3R)e^{Gp4}/TM3$, and viable, deficiency-bearing progeny were recovered). No recombinants were detected between members of other loci in this cluster (less than 0.05 map unit). This is in contrast to the intergenic distances to the left of ebony, where the intergenic distances average 0.1 map unit (J. MOHLER, unpublished results).

The *er1* complementation group has five members (*GC8*, *GC31*, *EC19*, *EC20* and *DC3*). All alleles are lethal, except *EC19*, which is a semilethal (approximately 10% normal viability).

The *er8* complementation group has five members. All of these alleles were generated with EMS, and most have simple complementation behavior. One mutant, *EC32*, is also mutant for ebony. This may be a double mutant, since in the mutagenesis in which the *EC32* mutation was generated one mutation was isolated for every 30 chromosomes screened. However, *EC32* is also semilethal over *In(3R)GC25*, which maps to the *er10* group in 93E, with heterozygous survivors having a Minute-like phenotype.

The *er11* complementation group consists of *EC36* and *EC38*. The *EC38* mutation is a visible mutation in combination with a deficiency: *EC38/Df* flies have warped opaque wings. The *EC36* mutation is lethal heterozygous to a deficiency, whereas *EC36/EC38* has warped wings.

The *er9* complementation group consists of *GC23* and *EC15*. The *GC23/EC15* heterozygote is fully viable but has roughened eyes like the *GC23/Df* heterozygotes and *GC23* homozygotes. Since *GC23* females have a more extreme phenotype than *GC23* males and *GC23* is an inversion from 93D to the centromeric heterochromatin, the lack of complementation with *EC15* may be due to position effect variegation. Variegation effects of *GC23* on the *er11* complementation group can be observed: some *EC36/GC23* flies have opaque wings reminiscent of the weaker *EC38* allele of the *er11* group, although this phenotype is not present in *EC38/GC23* or *GC23* homozygotes.

The er3, er19, er13 *and* er16 *complementation groups*: This group of four complementation groups is characterized by the failure to complement the *GC14* mutation, which thus appears to be a deletion, although there is no detectable cytological deficiency (MOHLER and PARDUE 1982). The *er3* complementation group is defined solely by the failure of complementation of *GC14* with $Df(3R)e^{Gp4}$. A low fraction of $GC14/Df(3R)e^{Gp4}$ heterozygotes survive to eclosion. These flies have warped wings and appear unhealthy. They are unable to walk or stand and die within a few days of eclosion. Thus, the gene(s) lost from this deficiency *trans*-heterozygote appear to be essential. It is interesting that these heterozygotes slightly resemble *EC38* in the neighboring *er11* complementation group and closely resemble *GC30* in the neighboring *er19* complementation group, although *Df(3R)GC14* complements the *EC38* mutation, and $Df(3R)e^{Gp4}$ complements the *GC30* mutation. The 93D heat shock locus has been mapped to this region [in the overlap of $Df(3R)e^{Gp4}$ and *Df(3R)GC14*; MOHLER and PARDUE (1982)].

The *er19* complementation group consists of the sole semilethal, *GC30*. This mutation complements $Df(3R)e^{Gp4}$; however, $GC30/Df(3R)e^{H4}$ and *GC30/GC14* individuals resemble $Df(3R)e^{Gp4}/GC14$ individuals. These flies survive to eclosion, possessing a warped wing phenotype, but, being unable to stand or walk, get stuck in the food soon afterward and are not generally recovered. The *er13* complementation group contains *EC40* and *EC43*. The *er16* complementation group is composed of *GC27* and *EC44*.

The er10, er12, er15, er17 *and* er18 *complementation groups*: Five additional complementation groups are mapped outside $Df(3R)e^{GC9}$ in 93E and 93F. These complementation groups are reasonably well separated by deficiency breakpoints.

The *er12* complementation group complements $Df(3R)e^{GC9}$ and $Df(3R)e^{F2}$ but does not complement $Df(3R)e^{F1}$. All of the alleles of *er12* are semilethal. *EC39* is the strongest allele: $EC39/Df(3R)e^{H4}$ has 5–10% normal viability. *EC35* and *GC32* are weaker alleles; over $Df(3R)e^{H4}$ they have 15–25% normal viability. The $er12/DF(3R)e^{H4}$ individuals all have longer developmental times, beginning eclosion after 14 days at 25° (as compared with 9 days for wild type). *EC35/EC39* and *GC32/EC39* individuals have reasonably good viability (about 40%

normal). In contrast, however, *EC35/GC32* appears to be lethal (less than 4% normal viability).

The *er10* complementation group appears to be a hot spot for induced mutagenesis; six of 25 mutations isolated by failure to complement $Df(3R)e^{H4}$ are members of this group. The *er10* complementation group complements $Df(3R)e^{F1}$, but not $Df(3R)e^{F3}$, and includes the lethal mutations *EC34, EC42, GC24, GC25, GC29* and *GC33*. The *er17* complementation group complements $Df(3R)e^{F3}$ but not $Df(3R)e^{D7}$. This group has two members, which are the lethals *GC28* and *DC4*.

Two complementation groups complement $Df(3R)e^{D7}$ but not $Df(3R)e^{H4}$. The *er15* complementation group consists of the single lethal mutation *DC2*. The *er18* complementation group consists of two mutations, *GC26* and *EC29*. The *GC26* mutation is a semilethal mutation, with approximately 5% viability. The *EC29* mutation is a homozygous viable mutation; these flies have warped wings (with incomplete penetrance) and are female sterile.

Heat shock response of the compound deficiency heterozygote ($Df(3R)e^{Gp4}$/Df(3R)GC14: Both $Df(3R)e^{Gp4}$ and *Df(3R)GC14* appear to be deleted for most or all of the 93D heat shock locus (MOHLER and PARDUE 1982). They both fail to puff at 93D under heat shock conditions and produce little or no RNA from 93D during heat shock. In addition, the 93D region of $Df(3R)e^{Gp4}$ chromosomes does not show detectable hybridization to heat shock RNA. In contrast, all of the lethal mutations that failed to complement $Df(3R)e^{Gp4}$ complemented *Df(3R)GC14* and vice versa. Therefore, the $Df(3R)e^{Gp4}$/*Df(3R)GC14* compound deficiency heterozygote is defective at both alleles for the 93D heat shock locus but retains one wild-type allele of all of the identified, flanking loci. Any difference in the heat shock response between $Df(3R)e^{Gp4}$/*Df(3R)GC14* deficiency *trans*-heterozygote and wild-type individuals is likely to be due to this defect in the 93D heat shock locus in the compound heterozygote. Since the $Df(3R)e^{Gp4}$/*Df(3R)GC14* compound deficiency heterozygote survives to adulthood, although rarely, it is possible to obtain material for experimentation.

Two components of the heat shock response of Drosophila were tested in these compound deficiency heterozygotes: the puffing response and the protein synthetic response. To test whether this deficiency *trans*-heterozygote produced the normal heat shock puffs, salivary glands of larvae from the $Df(3R)e^{Gp4}$/*TM3* × *Df(3R)GC14/TM3* cross were heat shocked at 38° for 15 min in MRM. The non-*TM3* larvae had major puffs at 63B, 67B, 87A, 87C and 95D. Minor puffs were observed at 33B, 48F, 50A and 70A. Except for the absence of a 93D puff (which is deleted), all of the normal heat shock puffs are present. Heat-shocked nuclei from larvae heterozygous for either 93D deficiency and *TM3* possessed these puffs as well as the 93D heat shock puff. Thus, the elimination of the 93D puff in this compound deficiency heterozygote produced no major changes in the pattern of heat shock-induced puffing.

Another feature of the heat shock response of *D. melanogaster* is a shift in the pattern of protein synthesis. Synthesis of a small number of proteins is rapidly induced, but synthesis of most other proteins is drastically reduced.

Upon return to 25°, synthesis of the previously synthesized proteins is resumed, whereas synthesis of induced proteins is slowly curtailed. The time for recovery of normal protein synthesis is variable and varies from tissue to tissue.

Ovaries from adult *Df(3R)e*^{Gp4}*/Df(3R)GC14* and Canton-S females were dissected out and placed individually in Eppendorf tubes. The Eppendorf tubes were suspended in a 37° water bath for 0.5 hr, and ^{35}S-methionine was added after 0, 1, 2 or 4 hr of recovery at 22°. An autoradiograph of polypeptides synthesized in these ovaries is shown in Figure 3. As is evident from this autoradiograph, no substantial difference between ovaries from Canton-S and *Df(3R)e*^{Gp4}*/Df(3R)GC14* females is observed at any time after the heat shock.

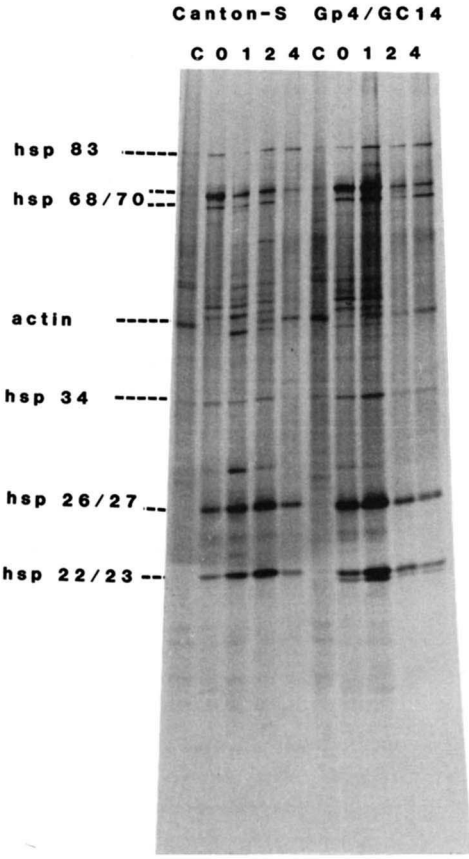

FIGURE 3.—Proteins synthesized during recovery from heat shock in flies heterozygous for two deficiencies for the 93D heat shock locus. Lanes marked C show proteins synthesized at 25° in wild-type Canton-S and in *Df(3R)e*^{Gp4}*/Df(3R)GC14*. Lanes marked 0, 1, 2 and 4 show proteins synthesized by ovaries heat shocked at 37° for 0.5 hr, then allowed to recover at 25° for 0, 1, 2 or 4 hr, respectively, and then labeled with ^{35}S-methionine for 0.5 hr. The recovery of 25° protein synthesis is most clearly reflected by the recovery of actin synthesis. The extra small heat shock protein seen in *Df(3R)e*^{Gp4}*/Df(3R)GC14* lanes is due to the fact that the *Df(3R)e*^{Gp4} chromosome bears a different allele of a *hsp22* polymorphism than that of the *Df(3R)GC14* or Canton-S chromosome.

In particular, all of the major heat shock proteins are present in heat-shocked *Df(3R)e^{Gp4}/Df(3R)GC14* ovaries, in quantities similar to those of heat-shocked Canton-S ovaries. No extra proteins are detected after a heat shock or at 25°. Recovery of actin synthesis resumes by 4 hr after the 37° treatment in both Canton-S and *Df(3R)e^{Gp4}/Df(3R)GC14* ovaries, by which time synthesis of the heat shock proteins is diminishing. Thus, no effect on the heat shock response at the level of gross protein synthetic differences was detected in this combination of deficiencies of the 93D heat shock locus.

Heat shock viability of the compound deficiency heterozygote (Df(3R)e^{Gp4}/Df(3R)-GC14): The results of MITCHELL *et al.* (1979) implicated the heat shock response in conferring viability following a heat shock. Although a brief (0.5 to 1 hr) 40° temperature shock was often sufficient to result in high mortality and phenocopy production, animals could be protected from the effects of this high temperature shock if they had been exposed briefly to a mild (33°) prior temperature shock. A 40° temperature shock usually results in the cessation of RNA and protein synthesis. In contrast, synthesis of heat shock proteins can be observed after a 40° temperature shock that was preceded by a mild temperature shock capable of induction of heat shock protein synthesis. MITCHELL and his co-workers proposed that the heat shock response was involved in the protection from the effects of severe temperature shocks. It was of interest to know whether the *Df(3R)e^{Gp4}/Df(3R)GC14 trans*-heterozygotes, which lack an intact 93D heat shock locus, are capable of protection from lethal temperature shocks.

Because the *Df(3R)e^{Gp4}/Df(3R)GC14 trans*-heterozygote has only very low adult viability (approximately 1%), it was necessary to test the effect of heat shock on viability at times prior to eclosion. Vials containing 1- to 5-day-old larvae (grown at 22°) from a cross between *Df(3R)e^{Gp4}/TM6B* and *Df(3R)GC14/TM6B* were given one of four heat treatments, and pupae were counted 5 days later. Pupae heterozygous for the *TM6B* chromosomes were distinguished from the deficiency *trans*-heterozygote (*Df(3R)e^{Gp4}/Df(3R)GC14*) by the presence or absence of the dominant pupal marker *Tb* (Tubby) carried on the *TM6B* chromosome.

The results, shown in Table 4, indicate that substantial numbers of the deficiency *trans*-heterozygotes survive in cultures given a heat treatment. Although the viability of larvae following a heat shock corresponds to the severity of the heat shock, the relative viability of the *trans*-deficiency heterozygotes compared with their *TM6B*-bearing sibs remains approximately constant (at approximately 27% of the *TM6B*-bearing larvae). In addition, preexposure to a mild 33° treatment before a severe 40° heat treatment results in an increase in viability of the *trans*-deficiency heterozygote. It appears, therefore, that the *Df(3R)e^{Gp4}/Df(3R)GC14 trans*-heterozygotes are equally affected by heat treatments as are *TM6B*-bearing larvae.

Because more than 70% of the *trans*-deficiency heterozygotes do not normally survive to pupation, it is possible that the 27% that survive the larval stages represent a subclass of the *trans*-deficiency heterozygotes that are more resistant to environmental insults such as heat shocks. Thus, the *trans*-defi-

TABLE 4

Viability of the Df(3R)e^{Gp4}/Df(3R)GC14 trans-*deficiency heterozygote following heat shock*

Treatment	Viability (±SE)	Relative viability (±SE)
NT	(1.00)	0.27 ± 0.05
0.5 hr 33°	1.53 ± 0.26	0.24 ± 0.06
0.5 hr 37°	0.86 ± 0.21	0.30 ± 0.09
0.5 hr 40°	0.12 ± 0.06	0.36 ± 0.25
0.5 hr 33° → 0.5 hr 40°	0.51 ± 0.10	0.20 ± 0.09

Vials containing 1- to 5-day old larvae from a cross between *Df(3R)e^{Gp4}/TM6B* females and *Df(3R)GC14/TM6B* males were given one of five heat shock treatments; the numbers of *TM6B* and non-*TM6B* pupae were counted 5 days later. (The five treatments were: NT, no treatment, 0.5-hr 33° heat shock, 0.5-hr 37° heat shock, 0.5-hr 40° heat shock and a 0.5-hr 33° heat shock followed by a 0.5-hr 40° heat shock.) The heat shock viability following a heat treatment is the ratio of the total number of pupae in the treated vials compared with the untreated vials. The relative viability of the *Df(3R)e^{Gp4}/Df(3R)GC14 trans*-deficiency heterozygotes is twice the ratio of non-*TM6B* pupae to *TM6B* pupae in the treated cultures.

ciency heterozygotes may be more sensitive to heat treatment than larvae with an intact 93D heat shock locus, but those heterozygous animals least resistant to heat treatment are most likely to die during larval development. However, despite this objection, these results demonstrate that an intact 93D heat shock locus is not necessary for survival following a mild heat shock or for protection from the effects of a severe heat shock by pretreatment with a mild heat treatment.

DISCUSSION

The 93D heat shock locus has been cytologically mapped on the basis of the ability of the 93D region of deficiency-containing chromosomes to: (1) puff in response to heat shock, (2) hybridize *in situ* with heat shock RNA and (3) incorporate ^{3}H-uridine into RNA during a heat shock (MOHLER and PARDUE 1982). These experiments map the locus within the small region of overlap between the distal breakpoint of *Df(3R)e^{Gp4}* and the proximal breakpoint of *Df(3R)GC14*. In the experiments reported here we have used the earlier mapping studies to design a screen for mutations in the 93D heat shock locus.

Mutagenesis with γ-irradiation, EMS and DEB has produced a total of 62 apparent point mutations defining 18 complementation groups (in addition to the previously known ebony locus) in the region between 93B11–13 and 93F6–8. At least one additional locus can be identified by the inability of *Df(3R)e^{Gp4}* and *Df(3R)GC14* to complement, for a total of at least 20 loci in this region. The deficiency *trans*-heterozygote (*Df(3R)e^{Gp4}/Df(3R)GC14*) retains little, if any, of the heat shock locus. These flies rarely survive to eclosion and are morphologically deformed and virtually inviable. This phenotype suggests that the heat shock locus contributes to normal viability, although it is possible that there are undetected genes lying in the region of overlap of the two deficiencies that are not represented among our apparent point mutations.

Since all of the apparent point mutations complement either *Df(3R)e^{Gp4}* or

Df(3R)GC14, none of the apparent point mutations map within the heat shock locus as defined by our cytological criteria. Our failure to detect point mutations in the heat shock locus has two possible explanations. First, the heat shock locus may have been missed due to a failure to saturate the region with mutations. Second, it is possible that the nature of the heat shock locus makes it refractory to the mutational scheme used, either because of its function (*e.g.*, it may be nonessential) or its structure.

Because mutations were isolated in screens involving two deficiencies, it is difficult to estimate the degree of saturation of the entire region. There are three subregions that have been screened at different intensities: (1) inside $Df(3R)e^{Gp4}$, outside $Df(3R)e^{H4}$, (2) inside both $Df(3R)e^{Gp4}$ and $Df(3R)e^{H4}$, (3) outside $Df(3R)e^{Gp4}$, inside $Df(3R)e^{H4}$. The most important region, which was screened most intensively, is the region of overlap between the two deficiencies, because it contains the heat shock locus.

A useful method for estimating the degree of saturation is to fit the distribution of mutations among complementation groups to a Poisson distribution. Because this statistical analysis assumes an equal probability of mutagenesis at each locus, when in reality mutation rates vary widely among loci, this method estimates the total number of loci with a similar mutation rate as those already identified. This Poisson-based estimate, therefore, yields a lower estimate of the number of missed loci, because loci with a lower mutation rate will not be included. An analysis of the mutations isolated that fail to complement both $Df(3R)e^{Gp4}$ and $Df(3R)e^{H4}$ shows that the distribution of mutations fits a Poisson distribution ($P = 0.56$). This analysis suggests that the chance of not having isolated mutations in any particular locus of similar mutability in this subregion is 0.05. The Poisson distribution analysis of the mutations of this subregion indicates that approximately 0.3 locus of similar mutability remains in this subregion. Therefore, it seems likely that either the 93D heat shock locus is less mutable, or mutations of the heat shock locus are less readily identifiable than those of the other genes identified here.

Estimation of the number of missed loci in the other subregions is more difficult. The leftmost subregion (inside $Df(3R)e^{Gp4}$, outside $Df(3R)e^{H4}$) is small enough that the four isolated, highly mutable loci probably represent most of the loci in this subregion. The rightmost subregion (outside $Df(3R)e^{Gp4}$, inside $Df(3R)e^{H4}$) is probably not near saturation: a Poisson fit suggests there are about 1.1 more loci in this subregion of similar mutability. There are likely to be more loci of lower mutability still undetected in this subregion.

The fact that the heat shock locus was not isolated in this mutagenesis screen suggests that the 93D heat shock locus may be less mutable than the surrounding loci. However, the large size of the transcribed region of the 93D heat shock locus (9.8 kb; LENGYEL *et al.* 1980) would indicate that the heat shock locus should have a large target size for mutagenesis, assuming that most of this region is of functional significance. One possible explanation is that this large locus may consist of multiple repeats and show little or no phenotypic change if only one repeat is defective. The 2-48BC heat shock locus of *D. hydei*, which is analogous to the 93D heat shock locus of *D. melanogaster* (LAKHOTIA and SINGH 1982), has been shown to contain multiple repeats of a

cloned cDNA sequence (PETERS *et al.* 1982). Unfortunately, neither the cloned cDNA nor *D. hydei* heat shock RNA hybridizes to 93D, so the studies in *D. hydei* cannot be confirmed directly in *D. melanogaster*. The fact that the only mutations that we have obtained that affect the heat shock locus are deficiencies is consistent with the idea that the locus contains sequence repeats.

It is possible that one or both of the two deficiencies, $Df(3R)e^{Gp4}$ and *Df(3R)GC14*, do not delete the entire heat shock locus, although no homology to heat shock RNA was detected in these deficiencies (MOHLER and PARDUE 1982). The sensitivity of the cytological techniques is limited, and a residual fraction (up to an estimated 25%) of the locus may have remained undetected. If the 93D heat shock locus consists of multiple sequence repeats, these deficiency chromosomes may retain one or more functional units of the heat shock locus. This leaves open the possibility that mutations mapping close to the ends of these deficiencies might be mutations in the heat shock locus that are complemented by a small amount of expression remaining in the deleted chromosome. At least two loci flanking what we have defined as the heat shock locus have phenotypes reminiscent of that of the compound deficiency heterozygote ($Df(3R)e^{Gp4}/Df(3R)GC14$). The *EC38* mutation of the *er11* locus has roof-like wings and fails to complement $Df(3R)e^{Gp4}$ but complements *Df(3R)GC14*. The *GC30* mutation of the *er19* locus is semilethal with survivors that have warped wings. Most *GC30/Df(3R)GC14* flies die soon after eclosion, as does the compound deficiency heterozygote; however, *GC30* complements $Df(3R)e^{Gp4}$. These mutations may represent subcompartments of the heat shock locus that are incompletely deleted in the two deficiencies used to create the compound deficiency heterozygote. Of course, many unrelated loci can give similar phenotypes, so the relationship between these loci will require more study.

Although we cannot be sure that the two deficiencies used to define the heat shock locus have removed all of the DNA sequence of that locus, sequences required in *cis* are definitely removed. Neither $Df(3R)e^{Gp4}$ nor *Df(3R)GC14* form the 93D heat shock puff when heterozygous with a wild-type locus. In addition, the *trans*-heterozygote of these two deficiencies does not puff during heat shock. Analysis of mutations in flanking complementation groups shows no evidence of involvement of these loci in the formation of the heat-induced puff. Chromosomes carrying mutations in *er19*, *er13* and *er16*, when heterozygous to *TM3*, all show induction of the 93D heat shock puff (J. MOHLER, unpublished results). The inversion, *In(3R)GC23*, can be used to physically separate the flanking loci to the left (*er11*, *er8* and *er9*) from the 93D heat shock puff. The puff forms just outside the inverted region in the normal place, whereas these loci have been moved adjacent to the chromocenter.

The uncertainty about the actual size of the 93D heat shock locus limits the conclusions that can be drawn from our studies of the $Df(3R)e^{Gp4}/Df(3R)GC14$ *trans*-heterozygote. However, these animals are clearly deficient for a significant amount of the 93D heat shock sequences and are unable to form a heat shock puff at 93D. It is clear, therefore, that the lesion formed in the $Df(3R)e^{Gp4}/Df(3R)GC14$ *trans*-heterozygote represents a major defect of the

93D heat shock locus. A test of the heat shock response in these animals reveals that the *trans*-heterozygote induces all of the normal heat shock puffs except 93D and produces the set of major heat shock proteins, followed by recovery of normal protein synthesis. In addition, *trans*-heterozygous deficiency larvae are capable of surviving 37° heat shocks and show increased resistance to severe 40° heat shocks if they are first subjected to a mild 33° heat shock to an extent similar to larvae bearing one wild-type 93D heat shock locus.

A major puff with the unusual features of the 93D puff is a component of the heat shock response in every Drosophila species that has been studied (LAKHOTIA and SINGH 1982). The phenotypic features of the puff thus show considerable evolutionary conservation throughout the Drosophila genus. In contrast, the sequence composition of this locus appears to be divergent between more distantly related species of Drosophila (PETERS, LUBSEN and SONDERMEIJER1980). The degree of evolutionary conservation at this locus within the Drosophila genus remains an open question, since at present the only assay for it is on polytene chromosomes. However, the existence of a similar locus in distantly related Drosophila species is indicative of a conserved function for the 93D heat shock locus.

If the 93D heat shock locus has a function, there are a number of possibilities why these studies have detected no effect in the heat shock response of a major lesion in this locus. The possibility that the *trans*-heterozygous deficiency animals retain some of the 93D sequence has already been mentioned. Flies may be able to survive the heat shocks used here with only a part of the locus. On the other hand, it should be pointed out that we have not assayed all aspects of the heat shock response. During the response, normal RNA metabolism is interrupted both in transcription and processing. Our experiments were not designed to test this aspect of the response. An apparently identical heat shock is induced by a wide variety of agents other than heat (reviewed by ASHBURNER and BONNER 1979). If the 93D locus were necessary only in the presence of certain inducing agents, we would not have detected an effect. Finally, it is possible that other loci in the genome can replace the function of the 93D heat shock locus. Attempts to isolate mutations in another heat shock locus, the repeated genes in 87A and 87C that encode the major 70,000-dalton protein, have also been unsuccessful (GAUSZ *et al.* 1979, 1981). Even when chromosomes known to retain only one functional copy of the gene were used for mutagenesis, no mutations that mapped within the locus were obtained (GAUSZ *et al.* 1981). It is likely that the related 68,000-dalton heat shock protein or the product of the 70,000-dalton cognate genes (INGOLIA and CRAIG 1982) can replace the function of the 70,000-dalton protein if the genes at 87A and 87C are nonfunctional. It may be that other aspects of the heat shock response have an equivalent backup mechanism.

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