# Genetic Analysis of the *Shaker* Gene Complex of *Drosophila melanogaster*

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ABSTRACT

The *Shaker* complex (*ShC*) spans over 350 kb in the 16F region of the *X* chromosome. It can be dissected by means of aneuploids into three main sections: the maternal effect (ME), the viable (V) and the haplolethal (HL) regions. The mutational analysis of *ShC* shows a high density of antimorphic mutations among 12 lethal complementation groups in addition to 14 viable alleles. The complex is the structural locus of a family of potassium channels as well as a number of functions relevant to the biology of the nervous system. The constituents of *ShC* seem to be linked by functional relationships in view of the similarity of the phenotypes, antimorphic nature of their mutations and the behavior in transheterozygotes. We discuss the relationship between the genetic organization of *ShC* and the functional coupling of potassium currents with the other functions encoded in the complex.

*SHAKER* was the first behavioral mutant detected in *Drosophila melanogaster* (CATSCH 1944). It was named after another mutant with a similar phenotype isolated earlier in *Drosophila funebris* (LUERS 1936). The original phenotype was described as the trembling of appendages in the anesthetized fly. The electrophysiological study of most viable *Sh* alleles has revealed a number of functional defects including: excess of neurotransmitter release at the neuromuscular junction (JAN, JAN and DENNIS 1977), abnormal action potentials in the cervical giant fiber (CGF) (TANOUYE, FERRÚS and FUJITA 1981; TANOUYE and FERRÚS 1985) and absence or altered kinetics of the fast voltage dependent transient $K^+$ current (Ia) in muscles under voltage clamp conditions (SALKOFF and WYMAN 1981; WU and HAUGLAND 1985) as well as in single channel studies of dissociated nervous systems (SOLC, ZAGOTTA and ALDRICH 1987). The subsequent molecular analysis of this locus has shown the existence of a complex transcription unit from which a large family of products is generated by means of differential and/or alternative splicing mechanisms (BAUMANN *et al.* 1987; TEMPEL *et al.* 1987; KAMB, IVERSON and TANOUYE 1987). Most of these products have structural features compatible with membrane proteins (PONGS *et al.* 1988; SCHWARTZ *et al.* 1988) and some of them have been demonstrated to function as $K^+$ channels after RNA expression in Xenopus oocytes (TIMPE *et al.* 1988; IVERSON *et al.* 1988). Different RNAs give rise to $K^+$ currents of different characteristics depending on the specific 3′ and 5′ exon combination present (KAMB, TSENG-CRANK and TANOUYE 1988). Thus, it seems that this transcription unit from *Shaker* encodes a variety of $K^+$ channels as well as a number of putative membrane proteins that do not appear to have the capability of generating, by themselves, gated ionic channels.

$K^+$ currents are known to be the most diverse class of ionic currents in terms of kinetics, pharmacology and sensitivity (HILLE 1984; RUDY 1988). Also, these currents are known to play an essential role in many aspects of the biology of organisms from morphogenesis (JAFFE 1979; KLINE, ROBINSON and NUCCITELLI 1983) to modulation of synaptic efficacy during learning (LEVITAN 1988; KANDEL and SCHWARTZ 1982). In addition and in contrast to other ionic currents, $K^+$ currents are ubiquitous among organisms, tissues and developmental stages.

*Shaker* was known to be a gene complex from the beginning of its genetic analysis (TANOUYE, FERRÚS and FUJITA 1981). We have entertained the hypothesis that the diversity of $K^+$ currents has its counterpart in the complexity of the *Shaker* locus. Now that one of the original propositions has been demonstrated, namely that *ShC* is the structural locus for a number of $K^+$ channels, it seems appropriate to dissect ShC into its genetic components, to study the biology of their mutations and to begin to unravel the functional relationships among these components. There are abundant examples in a variety of preparations illustrating how $K^+$ currents are modulated by means of phosphorylation, neuropeptides, cyclic nucleotides, etc. (LOGOTHETIS *et al.* 1987; NORTH *et al.* 1987; ASHCROFT 1988). Synaptic efficacy is largely based on the diversified modulation of $K^+$ channels (CROW 1988; LEVITAN 1988). In this context we will ask if the required functional coupling among these diverse activities is related to the genetic organization of ShC.

We do not define *Shaker* solely on the basis of the $K^+$ currents phenotype. Also, we do not assume that

the Sh products participate exclusively in the formation of ion channels. We find untenable such a one-to-one correspondence between gene products and biological features. Rather, we define a gene complex in broader terms as the portion of the DNA where clustered mutations show genetic relationships and/or similar phenotypes at any given level of observation.

## MATERIALS AND METHODS

**Mutants, rearrangements, mutagenesis and nomenclature:** The description of mutants and rearrangements used in this study can be found in LINDSLEY and GRELL (1968), LINDSLEY and ZIMM (1985, 1986, 1987) or TANOUYE, FERRÚS and FUJITA (1981). Also, Table 1 and Figure 1 show a summary of the rearrangements used.

The following agents were used as mutagens under the conditions of LEWIS and BACHER (1968) or AUERBACH and MOSER (1953): ethyl methanesulphonate (EMS) (250 mM), ethyl nitrosourea (ENU) (250 mM), formaldehyde (F), diepoxy butane (DEB) (50 mM) and X-ray (4000 r). Unless otherwise indicated, 4–7 days old $f^5$ *os* males were treated.

The T(*X*;*Y*)'s were induced by J. Merriam on a $y^+Y^S \cdot Y^L B^S$ chromosome. The proximal and distal elements can be obtained separatedly and are designated by superscript P or D. Males of the constitution $X/V7^P/Y$ are sterile due to the hiperploidy of the base of the *X*. We mutagenized $Y^SX \cdot Y^L$ compounds with X-ray in order to obtain deficiencies of the base of the *X* chromosome as fertile males $Y^SX \cdot Y^L*/V7^P$. In this way *Df(1)S4010* was obtained. *In(1)Px* (*Panoramix*) was obtained from the progeny of X-ray treated *os* males and detected by the dominant, larger than normal, eye size phenotype.

The mutations located within *ShC* are named after the region to which they map: ME, V or HL for *m*aternal *e*ffect, *v*iable and *h*aplo *l*ethal respectively, followed by the number of complementation group and the code number. For instance, $Sh^{MEI.305}$ indicates a *Shaker* mutation of the first group of complementation (from distal to proximal) in the ME region, isolated as code number 305.

**Characterization of the shaking activity.** The intensity of appendage vibration in the various genotypes listed in Table 5 was rated 1, 2 or 3 in increasing order. These estimations are based in the shaking activity of the mesothoracic legs of groups of ether-anesthetized (30 sec) flies. Care was taken to use flies not older than five days and not younger than one day. Aged *Sh* mutant adults (over 10 days) exhibit progressively more intense, although erratic, appendage vibration, possibly related to the conspicuous impairment of muscle structure observed in several *Sh* alleles. Also, very young *Sh* adults (1 day) do not express the shaking phenotype in full magnitude. The rating of the shaking activity of any given genotype was decided after the observation of a minimum of 20 flies from different crosses and observed during the entire process of recovery from ether anesthesia. Whenever possible, control sib adults were co-anesthetized with the mutant genotypes.

Assays with other anesthetics ($N_2$, $Cl_4C$, $CO_2$ and cold temperature) were carried out under the same conditions and yielded similar results as ethyl ether. However, anesthesia with triethylamine ("Fly Nap" Caroline Biological Supply) yielded results that differ among the *Sh* mutants (see results). The procedure of anesthesia with triethylamine was as follows: an impregnated brush was introduced in a 100-ml vial with 20 flies for 5, 3 or 1 min and the anesthetized flies were observed for 30 min or longer during recovery under the dissecting microscope. The following Sh viable alleles were tested: $Sh^5$, $Sh^{102}$, $Sh^{KS133}$ and $Sh^{rKO120}$.

**Electrophysiology:** Action potentials were registered from the CGF interneuron (KOTO *et al.* 1981). The procedure for dissection, experimental conditions and characteristics of the preparation can be found in TANOUYE and FERRÚS (1985).

**Mosaics:** Gynandromorphs were obtained from the progeny of $ca^{nd}/ca^{nd}$ females or among $R(1)2,In(1)w^{vc}/*$ embryos. The resulting mosaics were routinely analyzed for general behavior including possible shaking activity. Also, the extent of male/female territories were drawn on standard fly sketches. Each gynandromorph was either mounted in Euparal for detailed cuticular observation or processed for histology. A tentative fate map of the mutant focus of each lethal was calculated with the limited collection of gynandromorphs as described in HOTTA and BENZER (1972).

Somatic recombination clones were induced by X-ray (Philips MG 151 Be, 150 r/min, 100 kV, 15 mA and 2 mm Al filter at a total dose of 1000 r) in heterozygous larvae of the indicated age. Germ line clones were induced by the same procedure in *FS(1)KS1237 v/** larvae irradiated 24–48 h after egg laying. The use of this agametic dominant female sterile mutant (*FS(1)KS1237*) allows the detection of a germ line clone bearing female as a fly that under $CO_2$ anesthesia relaxes the genital muscles and liberates a retained egg. Under these conditions 6% of the irradiated control females carry a germ line clone (WIESCHAUS 1980). The development of each egg laid by these mosaic females was analyzed individually.

**Lethal phase and whole mounts:** Fecund females were allowed to lay eggs for 20-hr periods at 25° and 80% humidity on regular fly food plates containing 5% sucrose and a drop of live yeast. Eggs were collected, counted and transferred to petri dishes containing filter paper soaked in 5% sucrose and a few drops of yeast. Groups of 20–30 eggs were examined at 1-day intervals; thus, the fraction of dead individuals was estimated in 24-hr periods. Routinely, the lethal embryos were cleared and mounted for observation (VAN DER MEER, 1977). The classification of lethals as recessive, semidominant or dominant is according to HADORN (1955).

## RESULTS

**Aneuploid analysis:** In the process of analyzing the *ShC* we have used a number of chromosomal rearrangements (see Figure 1) with breakpoints in the vicinity of the *Shaker* locus. In order to identify their linear order and the existence of lack-of-function lethals, we constructed aneuploids in all feasible combinations. A summary of the information obtained is shown in Figure 1.

To dissect the 16EF region by means of aneuploids we constructed males $Df(1)JC153/W32^P$ and $Df(1)JC153/B55^P$. These males are viable (10–20%) with respect to their sib males $FM7c/W32^P$; *Dp(1;3)JC153/+*. We chose the latter as control because they correspond to the homologous gamete segregation in the females of the cross: ♀ *T(X;3)JC153/FM7c/TM6* × ♂ *W32/0*. A similar criterion was followed in the cross: ♀ $Df(1;3)JC153/FM7c/W32^P$; *Dp(1;3)JC153, Ki Sb/TM3* × ♂ *CS*. In practice,

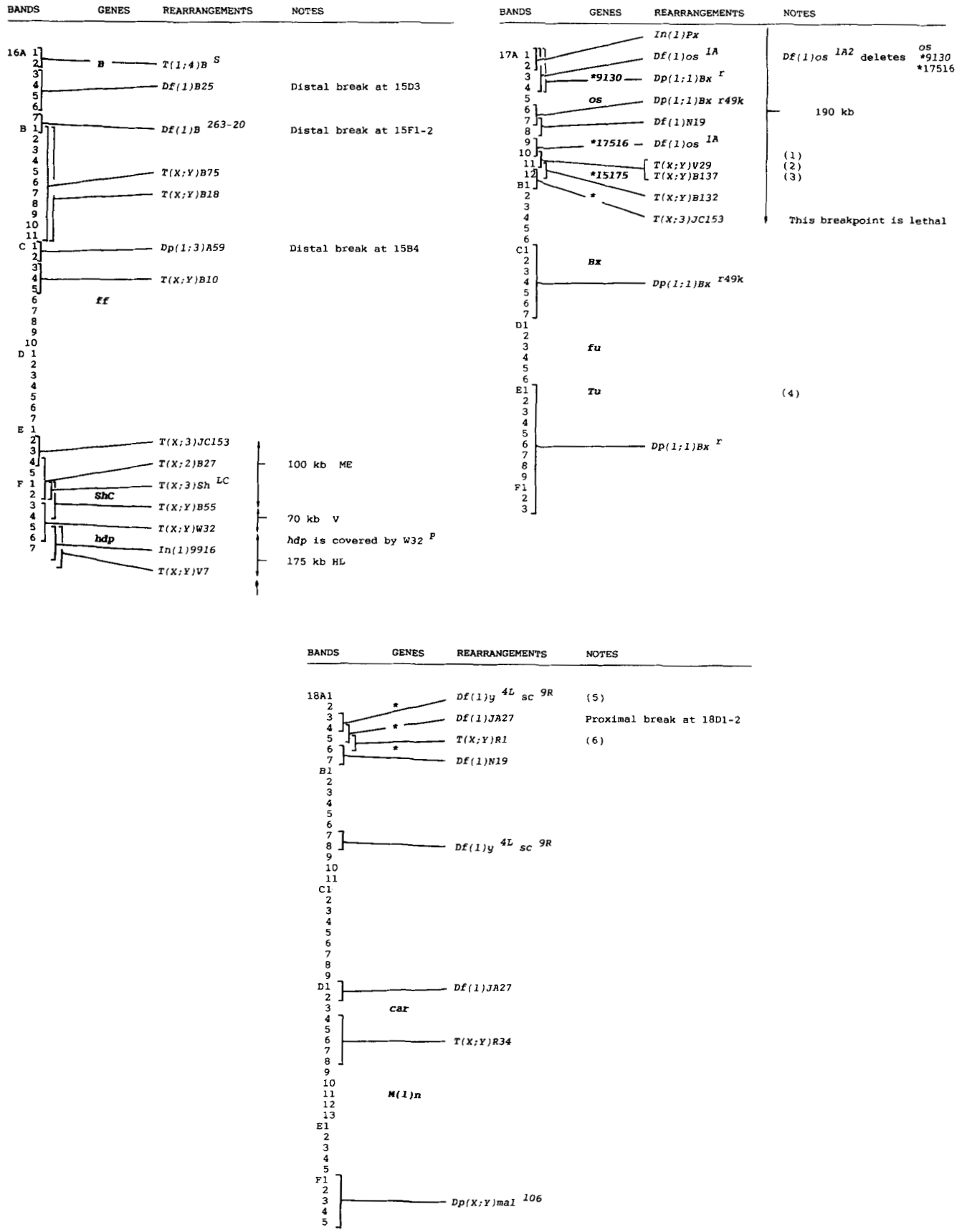



FIGURE 1.—Aneuploid analysis of the 16A-19A region. The entire DNA covered by the *Dp(1;3)JC153* has been cloned and the encompassed breakpoints have been located in the corresponding restriction fragments. The sterility of hyperploid males $X/X^P$ (from the T(X;Y)'s) is caused by the duplication of the 18D region. * = Predicted lack-of-function lethal. (1) $B137^P/Df(1)N19$ males are lethal. (2) The *V29* breakpoint is located 20 kb distal to *B137*. (3) $B137^D/V29^P$ or $B137^P/V29^D$ males are viable while $B137^D/B132^P$ are lethal. (4) $Tu/Dp(1;1)Bx^r$ and $Tu/Dp(1;1)Bx^{r49K}$ females are phenotypically $Tu^+$. (5) $R1^P$ does not cover $Df(1)y^{4L}\ sc^{9R}$. (6) $R1^D/Df(1)N19$ females and $R1^P/Df(1)JA27$ males are lethal.

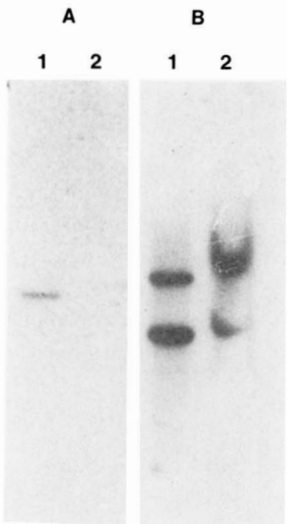



FIGURE 2.—ME⁻ males are viable. Southern blot of *Hin*dIII digested DNA from CS (lanes 1) and *Df(1)JC153,v/W32*$^{P}$; *TM1*/+ (lanes 2) males hybridized with probes from the ME region (panel A) and from outside the *ShC* (panel B). The probe used in panel A is the cDNA adm135H8 from coordinates −1.5 to 3.5. The probe used in panel B is lambda Bb5 clone from coordinates 313 to 331. The experimental males are deficient for the interval −76/−73 to 98/99.5 in the DNA map (Figure 3). Each lane contains the DNA extracted from a single male fly and both hybridizations were performed in the same blot.

the experimental as well as the control genotypes are scarce probably because other gamete segregations are favored. Molecular evidence for this deficiency was obtained in Southern blots of *Df(1)JC153,v/W32*$^{P}$ males probed with a DNA fragment from this interval (Figure 2). However, the viability of these deficient males is dependent on the maternal genotype. Attempts to generate these males from females *Df(1)JC153/FM7c/W32*$^{P}$ or *Df(1)JC153/FM7c/B55*$^{P}$ have failed. In these cases the maternal genotype was monosomic for the interval in question while in the crosses described above, the maternal genotype was euploid. Based on these observations, we named the interval JC153-W32 (Figure 3) as maternal effect region (ME), indicating that the nullosomy for this region is tolerated only if the normal (disomic) amount of products were supplied during the oogenesis. A further dissection of this region allowed to restrict the ME region to the interval JC153-B55. The viability of ME⁻ males implies the absence of lack-of-function lethal mutations in this interval (however, see below).

Adjacent and proximal to the ME region we define the viable region (V) on the basis of the viability of the aneuploid males *B55*$^{D}$*/W32*$^{P}$ as well as *B55*$^{P}$*/W32*$^{D}$ (Figure 3). These males are viable irrespective of the maternal ploidy for this region. As in the previous case, the viability of both aneuploids implies the absence of lack-of-function lethal mutations in this interval. The stronger phenotype of *B55*$^{D}$*/W32*$^{P}$ versus *B55*$^{P}$*/W32*$^{D}$ (Figure 4) indicates that the first aneuploid is the deficiency while the second is the corresponding duplication. The cloning of the DNA encompassing these breakpoints has proven this point (BAUMANN *et al.* 1987).

The aneuploid *W32*$^{D}$*/V7*$^{P}$ is lethal in males as well as in heterozygous females (Figure 3). The dominant lethality of this deletion can be rescued by one dose of the *Dp(1;3)JC153* or the fragment *W32*$^{P}$. We name this interval haplo lethal region (HL). This is the only HL region that we have detected between 15D and the centromere since females *V7*$^{D}$/+, *Df(1)B25*/+ and *B75*$^{D}$*/T(X;3)JC153/W32*$^{P}$ (see Figure 1) are viable.

For the purpose of the analysis of *ShC* it is important to note that the interval V7-JC153 proximal (14 bands) includes a minimum of three lack-of-function lethals since the aneuploid males *Df(1)N19/B137*$^{P}$, *B137*$^{D}$*/B132*$^{P}$ and *T(X;3)JC153* (see Figure 1) are not viable unless the additional fragment *V7*$^{P}$ is present. Also, it should be noted that the hyperploid males *X/Dp(1;3)JC153/Dp(1;3)JC153/B18*$^{P}$ are viable and do not show a shaking phenotype under visual inspection nor in the CGF action potentials (data not shown).

**Extent and constituents of the ShC:** Among the available rearrangements with breakpoints in the region (Table 1), *T(X;Y)B55*, *T(X;3)Sh*$^{LC}$, *T(X;Y)W32* and *T(X;Y)V7* show a noticeable shaking phenotype. Studies of $K^+$ currents on muscles under voltage clamp conditions have shown that *W32* eliminates Ia, while *B55* manifest a residual current and *V7* does not affect it (SALKOFF and WYMAN 1983). By contrast, *B55* is a more vigorous shaker than *W32* and *V7* is clearly distinguishable from the normal type (see Table 5 below). Also, the action potentials from the CGF interneuron are abnormal in the four cases (Figure 4) (see also TANOUYE, FERRÚS and FUJITA 1981). In agreement with the shaking activity, *B55* shows a more defective action potential than *W32* (Figure 4). The discrepancies between the phenotype definition at these three levels of observation probably reflects the diversity of biological functions encoded in the region. The nearest breakpoints without a shaking phenotype are those of *JC153* at the distal end and *S4010* at the proximal terminus (Figure 3). Thus, it can be said that the *ShC* is included within the interval defined by the latter two rearrangements.

The *Dp(1;3)JC153* covers about 22 bands. In order to identify the genetic components of the region where the *ShC* resides, we set out a lethal saturation experiment. Mutants were detected in a F2 screen and isolated over the *Dp(1;3)JC153* according to the procedure of Figure 5. This procedure allows the isolation of all loci mutable to lethality in this interval. The aneuploid analysis (see above) had indicated the existence of at least three lack of function lethals in the interval V7-JC153 proximal. Also, a dominant lethal would be expected in the HL region. Finally,

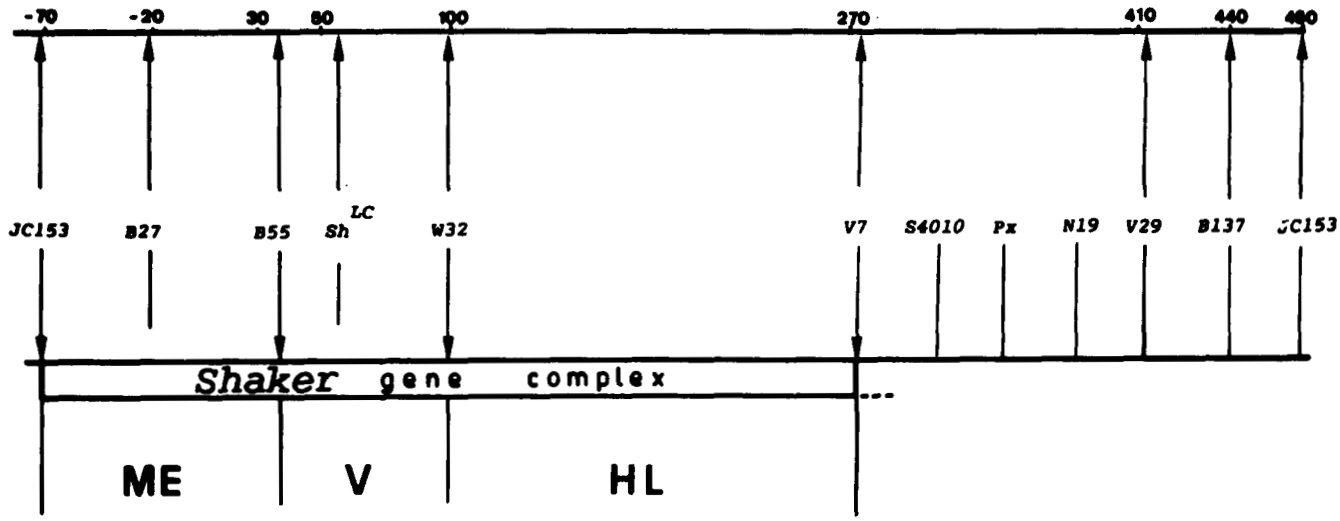



FIGURE 3.—Extent of the *Shaker* gene complex. The rearrangements used (see also Table 1 and Figure 10) are positioned with respect to their location in the map of the cloned DNA (upper scale). Those marked by arrowheads were used to dissect the region into three main zones: ME, V and HL. The proximal limit of *ShC* resides somewhere between *V7* and *S4010*.

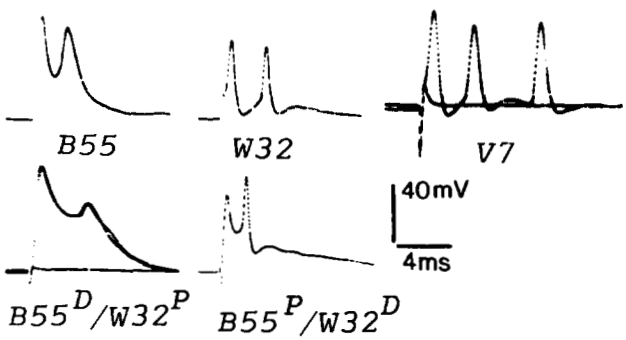



FIGURE 4.—Action potentials from rearrangements and aneuploids within *ShC*. Recordings were obtained from the CGF of males after brain stimulation. *V7* shows two traces, one of them below threshold. *Df(1)B55$^D$/W32$^P$* shows three traces, one of them below threshold. Note that *Df(1)B55$^D$/W32$^P$* has a longer repolarization delay than the *Dp(1)B55$^P$/W32$^D$* suggesting that a product(s) is encoded in this interval and its lack-of-function contributes to the abnormal profile of action potentials. Also, note that *Dp(1)B55$^P$/W32$^D$* shows a more severe phenotype than *W32* suggesting that the aneuploid perturbs the function of more products than it does *W32*.

the ME region should not contain lack-of-function lethals because of the viability of ME$^-$ males.

A total of 19902 fertile chromosomes were screened in the F2 generation after treatment with various mutagens (Table 2) and 33 lethals were isolated. These lethals were mapped with respect to the available breakpoints by constructing the aneuploids ♂ */W32$^P$*; ♂ */B55$^P$*; ♂ */V7$^P$*; ♀ */V7$^D$*; ♀ */Df(1)N19* and ♂ */V29$^P$*. The abundance of lethals within the *ShC vs.* the interval V7-JC153 proximal is a remarkable feature (Figure 6). It seems that the lethal mutational target of the JC153 distal-V7 region is much larger than the rest of DNA covered by *Dp(1;3)JC153*. Since the entire *Dp(1;3)JC153* has been cloned we can say that the 350 kb of *ShC* have yielded 29 lethals while the 190 kb outside of it (Figure 6) have yielded only 4 lethals in the saturation experiment. Thus, the ME, V and HL regions appear to house a high density of functions with respect to the remaining DNA covered by *Dp(1;3)JC153*.

The corresponding allelomorphism test among the isolated mutations revealed three complementation groups named MEI, II and III from distal to proximal, as components of the ME region (Figure 6). The finding of lethals in the ME region is an unexpected result in view of the viability of ME$^-$ males. In fact, this is the most frequently mutated region. None of the isolated lethals is rescued by extra doses of the ShC$^+$ in the maternal genotype. Indeed, the procedure of mutant isolation precludes the detection of mutations rescuable by maternal hyperploidy (Figure 5). On the other hand, since some of the alleles have been induced by X-ray, it is reasonable to assume that some of them are bona fide lack-of-function lethals (see DISCUSSION). It is evident that the lethality of these mutations is not a phenotype equivalent to their deletion. It can be concluded that the lethality of the ME mutations is an antimorphic trait (MULLER 1932) (see also below).

As expected, in the V region no lethals were found. In the HL region, the mutagenesis with alkylating agents, that usually produce point mutations, has failed to generate the expected dominant lethals. Only when X-ray was used, 3 dominant (DL) and 1 semidominant (SDL) lethals were found (Table 2). It seems that a major disruption of the genetic organization in HL is required to obtain dominant lethals. Also, while the ME region has three complementation groups, the HL region contains nine. However, both regions span over a similar length of DNA (Figures 3 and 6). The DL mutations can not be tested for allelomorphism because of their dominant lethality. It is not known if the three DL mutations correspond to the same com-

**TABLE 1**

**Rearrangements in the region of ShC**

| Chromosome | Breakpoints | Markers | Notes | References[a] |
|---|---|---|---|---|
| *T(X;Y)B75* | 16B/Y$^s$ | $X^P$,$y^+$; $X^D$,$y$ $B^s$ | Viable. Needs a *Y* chromosome | ♥ |
| *T(X;3)JC153* | 16E/17A12-B1 | *v* | Proximal breakpoint is lethal | ♣ |
| *T(X;2)B27* | 16E4-F2/36D-F | *B Sh*$^{KS133}$ | Viable although infrequent | ♠ |
| *T(X;Y)B55* | 16F1-4/Y$^s$ | $X^P$,$y^+$; $X^D$,$yB^s$ | Viable. Needs a *Y* chromosome | ♥ |
| *T(X;Y)W32* | 16F3-6/Y$^s$ | $X^P$,$y^+$; $X^D$,$y$ $w$ $f^1$ $B^s$ | Viable and fertile | ♥ |
| *T(X;3)Sh*$^{Lc}$ | 16F1-2/80 | *dnc*$^2$ | | ♠ |
| *T(X;Y)V7* | 16F5-8/Y$^s$ | $X^P$,$y^+$; $X^D$,$y$ $w$ $f^1$ $B^s$ | Viable and fertile | ♥ |
| *Df(1)S4010* | 17A/20 | $y$ $w$ $v$ $f^1$ | Induced on a *Y*$^s$*X.Y L* compound. Lost | ♠ |
| *In(1)Px* | 17A1-2/8C | *os* | *Panoramix* (*Px*) causes abnormally large eyes | ♠ |
| *Df(1)N19* | 17A7-8/18A6-7 | | | ♣ |
| *T(X;Y)V29* | 17A10-11/Y$^L$ | $X^P$,$B^s$; $X^D$,$y$ $w$ $f^1$ $y^+$ | | ♥ |
| *T(X;Y)B137* | 17A10-11/Y$^s$ | $X^P$,$y^+$; $X^D$,$y$ $B^s$ | | ♥ |
| *T(X;Y)B132* | 17A11-12/Y$^s$ | $X^P$,$y^+$; $X^D$,$y$ $B^s$ | | ♥ |

[a] ♥ = J. MERRIAM (personal communication); ♣ = J. LEFEVRE (personal communication; ♠ = this work.

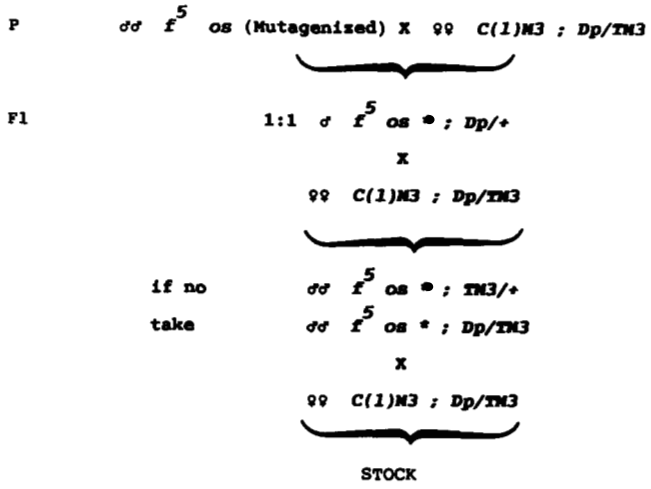



FIGURE 5.—Lethal saturation procedure. The *Dp(1;3)JC153* (abbreviated *Dp*) covers the entire *ShC* and three lack-of-function lethals proximal to *ShC* and *os* (see text and Figure 1). The mutagenized chromosomes are marked by *. Occasionally *os* males were used instead of $f^5$ *os*, but both chromosomes are of the same origin. Since the maternal genotype is hyperploid for *ShC*$^+$, no maternally rescuable lethals could be detected. The single male crosses of the F1 generation were screened for the absence of $f^5$ *os*; *TM3/+* indicating the presence of a lethal covered by *Dp(1;3)JC153*. Also, these crosses were inspected for visible mutants.

plementation unit nor if they are allelic to any of the other recessive lethals. Thus, the actual number of HL complementation groups could be higher.

Recombinational mapping among selected representatives of the complementation groups was undertaken (Figure 6). In general, the values of the frequencies of recombination are in agreement with the span of breakpoints and the DNA content known after cloning the area (Figure 3), roughly 1 cM = 1 Mb. The linear order of mutations in the ME and V regions was ascertained because the flanking markers in the heterozygous combination of mutants were different (see legend Figure 6). However, the linear order of most lethals within the HL region is not

**TABLE 2**

**Lethal saturation mutagenesis**

| Chromosomes[a] | Mutagen | Lethals | Code number |
|---|---|---|---|
| 2,855 | EMS | 13[b] | *162, 174, 305, 387, 581, 583, 598, 1199, 1359, 1579, 1614, 1929, 2215* |
| 1,166 | ENU | 5 | *459, 484, 2288, 2496, 3014* |
| 502 | DEB | 0 | |
| 1,462 | F | 0 | |
| 13,917 | X-ray | 14 | *254, 2270, 4058, 5051, 7688, 8384, 9130, 9916, 12748, 13167, 13193, 15175, 17053, 17516* |
| | Spontaneous | 1 | *17266*[c] |
| 19,902 | | 33 | (29 of them within ShC) |

[a] The number of sterile chromosomes (average of 21%) has been deducted.

[b] The frequency of mutability of *ShC* (about $4.5 \times 10^{-3}$) can be compared with that of *yellow* = $1.7 \times 10^{-3}$ or with that of *rosy* = $1.5 \times 10^{-3}$.

[c] *17266* is a gift of A. SCHALET.

The mutations *5051*, *9916* and *13193* are dominant lethals; *8384* is semidominant and the rest are recessive (however see also Figure 7). EMS, ethylmethanesulfonate; ENU, ethylnitrosourea; DEB, diepoxybutane; F, formaldehyde.

known. The case of *174* and *1614* is particularly interesting. These two mutations belong to different complementation groups and can be separated by recombination. The exchange of flanking markers in the recombinants unequivocally locates *174* distal to *1614* (see legend of Figure 6). However *174* fails to complement *4058* which is located adjacent to *V7* according to Southern blot analysis (I. KRAH-JENTGENS *et al.*, submitted for publication). Because elements at both extremes of the HL region fail to complement, the whole region must be considered as a functional unit.

As a test for detecting possible rearrangements

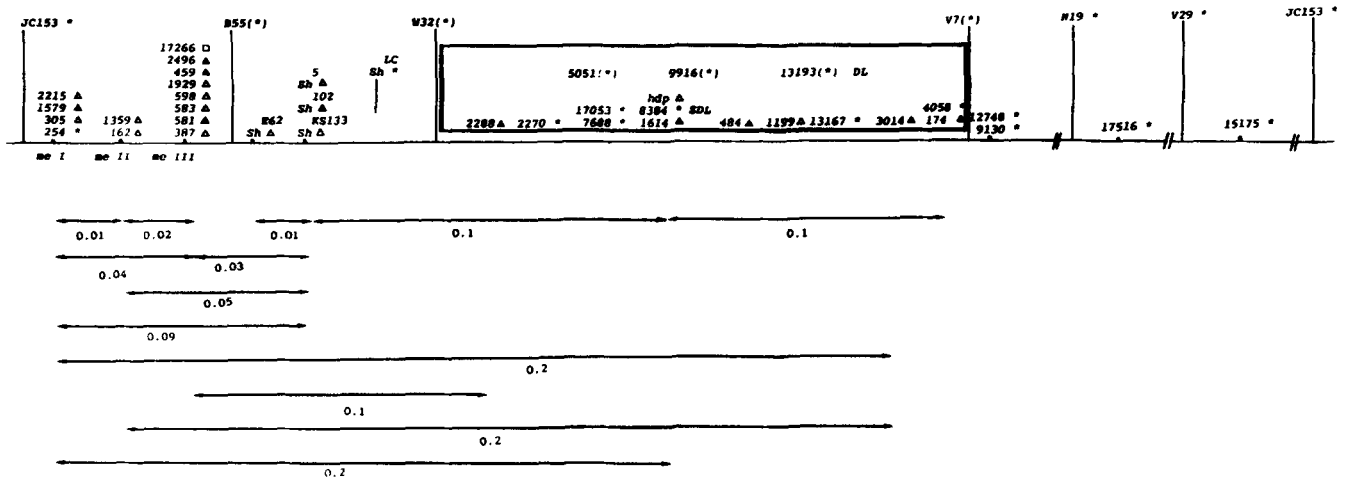



FIGURE 6.—Mutant saturation of the *Sh* region. Lethals are marked by a code number and the mutagen of origin by the symbols: Δ = EMS; ▲ = ENU; □ = spontaneous or • = X-ray. The linear order of ME lethals is based on the exchange of flanking markers in the recombinants obtained. The most frequent combination of flanking markers used was: *y w* $f^{36a}$*/$f^{5}$* *os*. The linear order of the HL lethals (box) has not been determined except for *174/1614*. The dominant lethals (DL) *5051*, *9916* and *13193* can not be adscribed to a complementation group because of this feature and show 0 viability in heterozygous females. The semidominant lethal (SDL) *8384* is poorly viable (about 30%) in heterozygous females and show 0 viability over *1614*. Also, it shows a wings up phenotype over *hdp*. The recombination frequencies are based on a minimum of two recombinants after pooling data from several crosses. It should be pointed out that this is an "attempt" of mutant saturation. It is not possible, at this time, to know if all existing complementation groups have been identified nor if all existing transcriptional units have been mutated.

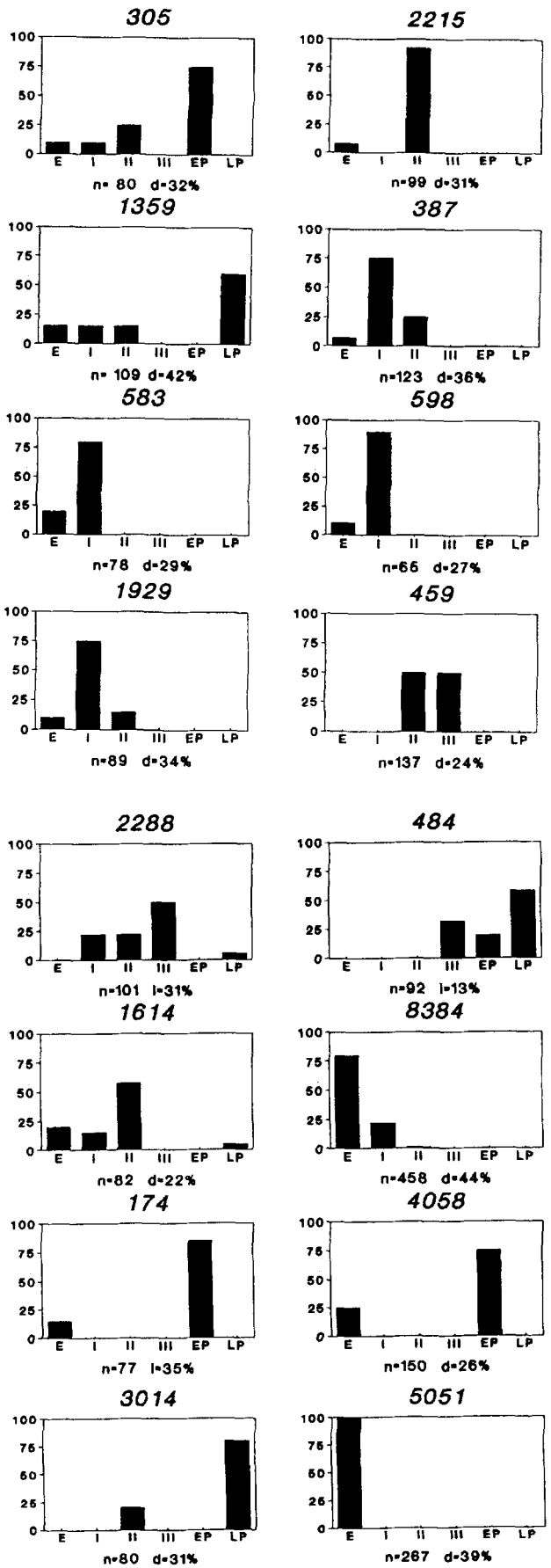
among the isolated lethals we screened the salivary gland chromosomes (*2215*, *459*, *2288*, *3014*, $8384^{SDL}$, $9916^{DL}$, $5051^{DL}$, and $13193^{DL}$) or measured the frequencies of recombination between flanking markers (*305*, *1359*, *387*, *1929*, *174*, *484*, *1614*, *2288*, and $5051^{DL}$). It was found that $9916^{DL}$ is an inversion with breaks at 16F and 14A. *2288* seems to be a very small deletion of the proximal part of 16F. This mutation reduces the frequency of recombination sixfold between *ff* and $Sh^{KS133}$. Also, $5051^{DL}$ reduces by 30% the recombination in the interval *f-os* suggesting that, although not detected by us in the polytene chromosomes, this mutation might be a subtle rearrangement. The rest of mutations tested do not alter the chromosome banding nor the recombination frequency.

**Biology of ShC mutants:** Characterization of the isolated mutations consisted in the study of the following traits: CGF action potentials, lethality phase and mosaic analysis. The types of mosaics studied include gynandromorph, somatic and germ line clones.

*The ME mutants*: The MEI group is defined by the mutations *305*, *254*, *1579* and *2215*. The study of *305* and *2215* shows that, although both mutations belong to the same complementation group, their lethal phases are different (Figure 7) suggesting that several functions might be encoded in this complementation group. Also, *2215* complements only partially the adjacent mutation *1359*. The corresponding heterozygous females show a Minute-like phenotype. The MEI mutations are lethal in gynandromorphs (Table 3). We attempted to generate smaller mosaics by means of somatic recombination in heterozygous larvae $f^{5}$ * *os*/*y w* irradiated at 48–72 or 24–48 h before puparium formation. No mutant spots were recovered while 11 notum, 9 eye and 34 abdomen control twin *y w* clones were found, indicating that *305* and *2215* are cell lethal mutations (RIPOLL 1977; BRYANT and ZORNETZER 1973). Similarly, the attempts to generate germ line mosaics (see Material and Methods) have failed (Table 4). The cell lethal condition of these mutations precludes any further characterization.

The MEII group is defined by the mutations *162* and *1359*. The latter was studied in detail. The major component of the letal phase occurs at the late pupal period (Figure 7), however no adult escapers have ever been detected. The lethality appears to have a slight dominant component since the fraction of lethal individuals in an outcross is higher than the expected 25% (Figure 7). The mutant territory in gynandromorphs shows defective bristle and vein patterns. Also, the mutant region of these mosaics exhibits a noticeable shaking activity (Table 3). Finally, the germ line clones are obtained in a normal frequency although the resulting embryos fail to develop irrespective of the zygotic genotype (Table 4). These abortive embryos do not progress beyond 40% embryogenesis in contrast with the relatively late lethal phase observed in regular outcrosses (Figure 7) (see DISCUSSION). Since 50% of these embryos should be *1359*/+ females, it can be concluded that the oogenesis from a *1359/1359* oogonia is defective for an essential maternal function which can not be supplied by the normal genome of the zygote.

The MEIII group is defined by eight mutations




(Figure 6). We have studied *387*, *583*, *598*, *1929* and *459* as representatives of the group. Their lethal phases are very similar in all cases (Figure 7) with the possible exception of *459*. The latter exhibits a clear dominant shaking activity in heterozygotes. However the inspection of polytene chromosomes, measurement of recombination frequency between flanking markers and Southern blot analysis has yielded no evidence for a rearrangement that could affect simultaneously the ME and V regions. Similarly, *387* exhibits a mild to weak shaking activity in heterozygous females. In this case, we registered the CGF action potentials and found them to be abnormal (Figure 8) resembling some viable *Sh* alleles (see below). For the gynandromorph analysis we used *1929*. The 16 mosaics obtained show normal hypodermal differentiation. However, 12 of them had at least one leg abnormally positioned or with shaking activity (Table 3). The germ line mosaics (Table 4) from the five alleles tested indicate that the mutated MEIII function(s) do not alter oogenesis. An exceptional case, however, is *1929*. In this case the homozygous ovarioles yield viable mutant males (Table 4). Under the usual maternal genotypes (**/FM6*) as well as in outcrosses (**/+*) these mutations show complete lethality. It appears that, when oogenesis is carried out by cells containing a mixture of mutated and normal products, which is the case of heterozygous mothers *1929/+*, the maternal effect on the development of male *1929* zygotes is more deleterious than when the cells contain only mutated products. This phenomenon is indicative of the antimorphic nature of the mutation and also a suggestion of the involvement of MEIII function(s) in oogenesis. Furthermore, it calls for a mechanism by which the normal MEIII products give rise to multimeric structures (see DISCUSSION).

Finally, the ME region harbours also the site of the viable rearrangement *T(X;2)B27* (Figure 3). This mutation was induced in a $Sh^{KS133}$ chromosome and detected as a modifier of the shaking phenotype. In the double mutant, the shaking activity is of a higher frequency and amplitude than in $Sh^{KS133}$. Since the breakpoint of this translocation in chromosome *II* is in the centromeric heterochromatin (Table 1), the dominant enhancing effect is most likely due to the functional modification of the *X* chromosome component. The subsequent study of the CGF action potentials of this double mutant show the typical

FIGURE 7.—Lethal phase of *ShC* mutants. A total of *n* eggs from outcrosses ♀ */+ × ♂ +* was followed (see MATERIAL AND METHODS) during development and the total percentage of dead individuals (d) was distributed in the corresponding instars. Mutants in the upper half of the figure belong to the ME region and those in the lower half belong to the HL region. Note that some lethals (*1359*, *387*, *8384* and *5051*) exhibit significant dominance in this trait. E = embryo, LI-III = larval stages, EP = early pupae, LP = late pupae.

**TABLE 3**

**Gynandromorph analysis**

| Region | Mutant | *n* | Control | Shaking activity | Hypodermal phenotype |
|---|---|---|---|---|---|
| ME | *305* | 0 | 46 | | |
| | *2215* | 0 | 32 | | |
| | *1359* | 18 | 52 | ++ | Bristles short, disoriented and fewer; plexated veins |
| | *1929* | 16 | 61 | ++ | Normal; abnormal position of wings and legs |
| HL | *1614* | 14 | 23 | ++ | Normal; abnormal position of wings and legs |
| | *174* | 18 | 174 | | Very few and short bristles. Gynandromorphs with small male patches |
| | *2288* | 13 | 43 | + | Bristles short, disoriented and fewer; plexated wings |
| | *3014* | 23 | 35 | − | Bristles short; sluggish |
| V | $Sh^{KS133}$ | 70 | 64 | +++ | Normal |

Mosaics were obtained from the crosses: ♀ $Sh^{KS133}/FM6$; $ca^{nd}/ca^{nd}$ × ♂ *y w* and ♀ $f^5$ *os* /*FM6* × ♂ *R(1)2*, $In(1)w^{vC}$. *n* = experimental mosaics. Control = ♀*FM6/y w* or ♀ FM6/*R(1)2*, $In(1)w^{vC}$ mosaics. Shaking activity refers to any appendage. The fate map of this behavior (HOTTA and BENZER 1972) indicates independent foci for each leg and a ventral location (data not shown).

**TABLE 4**

**Germ line mosaic analysis**

| Sh region | Lethal | *n* | Clones | Notes |
|---|---|---|---|---|
| MEI | *305* | 376 | 2[a] | Germ line lethal |
| | *2215* | 319 | 0 | Germ line lethal |
| MEII | *1359* | 259 | 21 | Eggs do not develop independently of the zygotic genotype |
| MEIII | *387* | 130 | 9 | Zygotic lethal |
| | *583* | 38 | 4 | Zygotic lethal |
| | *598* | 210 | 9 | Zygotic lethal |
| | *1929* | 369 | 14 | 11 clones yield 10% *1929* adults |
| | *459* | 90 | 3 | Zygotic lethal |
| HL | *174* | 208 | 1[a] | Germ line lethal |
| | *1614* | 344 | 19 | 10 clones yield 14% *1614* adults |
| | *484* | 182 | 8 | 7 clones yield 25% *484* adults |
| | *3014* | 207 | 11 | Zygotic lethal |
| | *2288* | 217 | 1[a] | Germ line lethal |

[a] Recombinant took place distal to the lethal locus as proven by the analysis of the offspring. Viability of the lethal escapers is calculated with respect to the sib females *FM6/**. The escapers lived for a few days with progressive motor impairments and failed to mate. *n* = number of irradiated *FS(1)KS1237,v/f*$^5$ * *os* females screened (see MATERIALS AND METHODS).

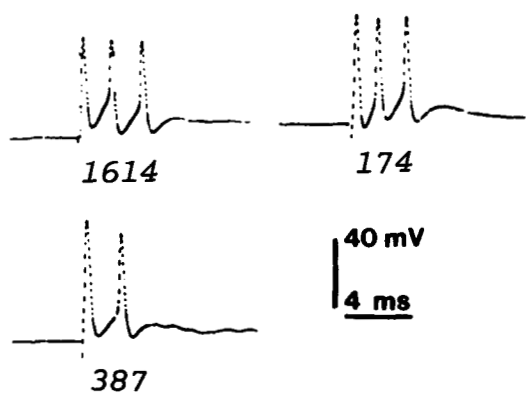



FIGURE 8.—Abnormal action potentials from *ShC* lethals. Recordings were obtained from the CGF of $f^5$ *1614 os/B137*$^D$; *y w* $f^{36a}$ *174/FM6* and $f^5$ *387 os/FM6* females. Similar results were obtained in $f^5$ *1614 os/FM6*. Since the chromosomal background of origin and the *X* homolog are different in these genotypes, the possibility that an unknown dominant factor be the cause of the abnormal action potentials appears very unlikely.

abnormal profile of action potentials from $Sh^{KS133}$ (data not shown). Thus, it seems that the modification of the shaking activity due to the B27 breakpoint occurs downstream from the biological functions involved, perhaps at the neuromuscular level.

In summary, mutations of the ME region affect, in different ways, oogenesis; some of them affect also the differentiation of the hypoderm and/or the physiology of the CGF. The normal function(s) of MEIII possibly requires the formation of multimers. Also, the viability of ME$^-$ males demonstrates that all these mutations are antimorphs.

*The V mutants*: The V region is defined by the breakpoints of B55 and W32. Based on gene dosage experiments we had located some viable *Sh* alleles in this interval (TANOUYE, FERRÚS and FUJITA 1981). Since the mutations in this region are viable, the behavioral and electrophysiological analyses have been more feasible. The shaking activity is not dependent on the state of anesthesia nor the anesthetic used. Flies treated with $CO_2$, $Cl_4C$, $N_2$ or cold temperature (4°C) vibrate their appendages with the same characteristics as when they begin to recover from ether anesthesia. Also, fully awake flies manifest chronic vibration as well as abnormal action potentials. The only differential effect detected was with triethylamine. The mutant $Sh^{rKO120}$ seems to be hypersensitive to this agent and treatments (see MATERIALS AND METHODS) that cause recoverable anesthesia in other strains, are lethal to this allele. With 1 min of treatment, 60% of $Sh^{rKO120}$ flies die. In general, the stronger the shaking activity of the allele the less deleterious is the anesthetic treatment with triethyla-

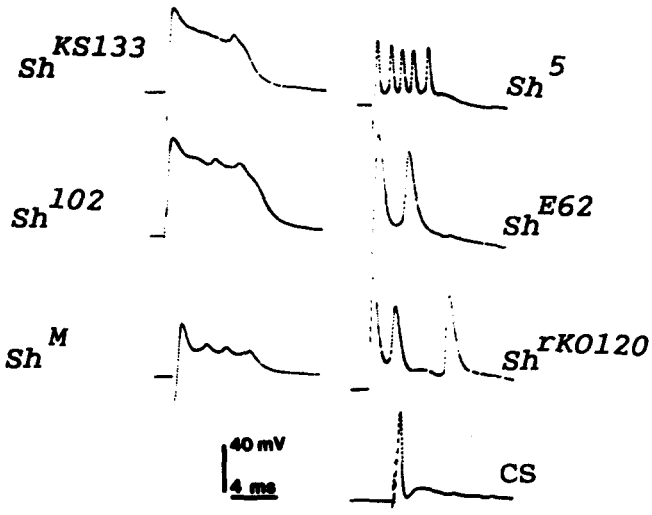



FIGURE 9.—Allele specificity of abnormal action potentials from viable mutants of *ShC*. Note the two types of abnormal profiles (left *vs.* right columns).

mine. Other cases of lethal effects with this agent are described in TANSEY *et al.* (1987).

There is a clear allele specificity in the appendage vibration and the action potential abnormality (Figure 9). We attempted to map by recombination viable *Sh* alleles with similar or different action potential characteristics. A pair of alleles ($Sh^{KS133}$ and $Sh^{E62}$) with different phenotypes showed to be separable by recombination (Figure 6). By contrast, the case of $Sh^{KS133}$ and $Sh^{102}$ with similar phenotypes yielded no recombinants among 8544 male offspring. However the case of $Sh^{KS133}$ and $Sh^{5}$ with clearly different phenotypes showed no recombinants among 10112 male offspring. The existence of recombination is a definitive proof of a relatively distant location of the mutations. However, the lack of recombination can be due to either the close proximity of the alleles in question or the existence of a subtle rearrangement in one of them causing defects in chromosome pairing. In the latter case, the Southern blot analysis should serve to detect such rearrangements. Since no DNA restriction pattern abnormalities have been detected in $Sh^{KS133}$ and $Sh^{5}$ flies (data not shown), it is suggested that these might be closely linked point mutations.

The shaking phenotype is autonomous in mosaics. We generated gynandromorphs by crossing ♀ $Sh^{KS133}$/*FM6*; $ca^{nd}/ca^{nd}$ × ♂ *y w*. A total of 70 experimental mosaics were obtained. The individual inspection of these mosaics showed that the shaking of any given appendage is independent from that of its contralateral homolog and is associated with the mutant condition of the corresponding hypodermal landmarks. It should be noted that in homozygous *Sh* mutants the intense vibration of appendages shows a bilateral coordination. However, in these mosaics and certain states of recovery from anesthesia in the homozygous flies, it can be seen that the shaking activity is autonomous for each appendage. A further proof of the autonomy of the shaking phenotype is observed when an appendage is severed from a mutant fly. Under this drastic condition, the severed leg continues to vibrate for several minutes decaying slowly. It should be noted that the severed leg contains no motorneuron somata, only nerve terminals and muscles, thus it can be said that the shaking activity is autonomous to the axon-muscle structures.

*The HL mutants*: The HL region is defined by the W32 and V7 breakpoints. A total of 16 mutations (Figure 6) have been mapped to this region. Allelomorphism indicates the existence of at least nine complementation groups. Since the dominant lethal mutations do not allow any allelomorphism test, the actual number of complementation groups might be even higher. There are reasons (see below) to believe that all functions coded in the HL region are functionally related. Therefore, we will describe the biology of HL mutants as a single group.

As with the MEIII mutant *387*, we noticed that *174*/+ and *1614*/+ adults have a visible shaking activity. The corresponding CGF action potentials show also an abnormal profile (Figure 8), suggesting that these functions are similar to those coded in the V region. For the gynandromorph analysis we used the mutations *1614*, *174*, *2288* and *3014* (Table 3). All of them are visible (about 50% versus control) in these mosaics although *174* yield very few cases (10% versus control) and with small disperse patches of mutant territory. Among the mosaics obtained, abnormal position of appendages and movements that could possibly be related to neuro/muscular defects were detected in *1614* and *3014* cases. The limited collection of gynandromorphs obtained allowed us to calculate tentative fate maps of the lethality foci (data not shown). In all cases (including the ME mutations), these are located in the ventral side of the embryo, roughly the anlage where the CNS and muscles originate (POULSON 1950). Also, the viable mutation *held up* (*hdp*) has been located within the HL region (HOMYK and EMERSON, 1988). We have found it to be allelic to *1614* and *8384* with respect to the muscle phenotype. However, abnormal wing position is a common feature of aged individuals of the genotypes: ♀ *1614*/+, ♂ $Sh^{5}$, ♂ $Sh^{120A}$, ♂ *V7* and ♂ *B55*. It is not known if these defects are of muscle or neural origin.

For germ line mosaics we used the mutations *174*, *1614*, *484*, *3014* and *2288* (Table 4). With the exception of *3014*, all the mutations tested seem to alter oogenesis. Two of them (*174* and *2288*) causing lethality of the oogonia and another two (*1614* and *484*) causing the same phenomenon as the MEIII mutant *1929*, namely the homozygous ovarioles give rise to mutant survivors (Table 4). Here too, these males

died within a few days after eclosion showing a progressive decay of their motor activity.

The HL region was tested for its dominant haplo lethality in somatic mosaics in *Df(1)JC153/FM6*; *Dp(1;3)JC153, Ki Sb*/+ larvae irradiated 72–96 h after egg laying. A total of 4 notum and 13 abdomen $Ki^+$ $Sb^+$ clones were obtained indicating that the $HL^-$/+ condition is tolerated in hypodermal clones. Also, the irradiation of $W32^D/V7^P$; *Dp(1;3)JC153 Ki Sb*/+ larvae yielded 2 clones in the notum (maximum size 5 bristles) and 11 clones in the abdomen (maximum size 4 bristles), in all cases without detectable abnormalities. These results indicate that the $HL^-$ condition is also cell viable in the hypoderm. The contrast between the abnormal morphology of mutant hypoderm in the gynandromorphs of *174*, *2288* and *3014* and the lack of defects in $HL^-$ hypodermal spots, indicates that this trait of the mutant phenotype is not equivalent to the corresponding deletion. In addition, it points towards the antimorphic nature of these mutations.

In germ line mosaics, the condition $ME^-$ $HL^-/ME^-$ $HL^+$ (genotype: $Df(1)JC153/Df(1)JC153/W32^P$) is lethal (0 clones in 152 females screened). Also, the condition $ME^-$ $HL^-/ME^+$ $HL^+$ (genotype: $Df(1)JC153/T(X;3)JC153$) is lethal (0 clones in 512 females screened). These experiments indicate that the HL region encodes dominant lack-of-function (*i.e.*, equivalent to their deletion) properties required during oogenesis. That is, a dominant female sterile mutation would be expected in the mutant saturation of this region. Possibly, the dominant lethal mutations *5051*, *9916* or *13193* would fulfill this expectation, albeit their dominant lethality prevents the testing of this possibility.

The HL region also includes the viable rearrangement *T(X;Y)V7* which shows shaking and abnormal CGF action potential phenotypes (Figure 4). The aneuploid ♀ $V7^D$/+ is viable although with a *Minute* phenotype due to the haploinsufficiency of the $M(1)n^+$ locus. Also, we questioned to which element (distal or proximal) is the dominant shaking phenotype associated?. Table 5 shows the degree of shaking activity of various aneuploids (see also Figure 10). It seems that the dominant shaking phenotype is associated with the distal as well as the proximal elements of *B55* and *W32*. In *V7*, only the distal element causes dominant shaking.

**Phenotypes of *trans*-heterozygotes:** Since the electrophysiological analysis of most of the ME and HL lethals is still pending, we searched for more genetic evidence for functional relationships among the isolated mutations. For that purpose we constructed all possible pairwise combinations of lethals in heterozygous females which were then crossed to *FM6* males. In each cross we screened for the appearance of escaper males of each lethal involved. Table 6 shows

**TABLE 5**

**Shaking activity of mutants and aneuploids**

| Genotype | Sh activity |
|---|---|
| $Sh^{KS133}$ | 3 |
| $Sh^5$ | 3 |
| $Sh^{102}$ | 3 |
| $Sh^M$ | 2 |
| $Sh^{E62}$ | 3 |
| $Sh^{rK0120}$ | 2 |
| *B55* | 3 |
| *W32* | 2 |
| *V7* | 1 |
| $FM7a/W32^P$ | 1 |
| $FM7a/B55^P$ | 1 |
| $FM7a/W32^P/B55^P$ | 1 |
| $B55/W32^P$ | 3 |
| $V7^D/W32^P$ | 2 |
| $V7^D/B55^P/V29^P$ | 1 |
| $V7^D/B55^P$ | 2 |
| $W32^D/B55^P$ | 2 |
| $B55^D/W32^P$ | 3 |
| $B55/V29^P$ | 3 |
| $V29^D/W32^P$ | 1 |
| $Sh^{KS133}/W32^P$ | 3 |
| $FM7a/V7^D$ | 2 |
| *B55/FM7a* | 2 |
| $B55^D/W32^P/FM7b$ | 3 |
| $W32^D/FM6/B75^P$ | 1 |
| $W32^D/B75^P$ | 2 |
| $B75/W32^P$ | 1 |
| $XX/W32^P$ | 1 |
| $B75^D/W32^P/FM7b$ | 2 |
| $B55/V29^D$ | 2 |
| $B55/V29^D/W32^P$ | 2 |
| $Df(1)JC153/FM7c/B55^P$ | 2 |
| $Df(1)JC153/B55^P$ | 1 |
| $Df(1)JC153/FM7c/W32^P$ | 2 |
| $Df(1)JC153/W32^P$ | 2 |
| $B55^D/FM6/Dp(1;3)JC153$ | 2 |
| $B55^D/B18^P$ | 2 |

The intensity of shaking activity is rated 1 to 3 in increasing order. Note that $ME^-V^-$ ($Df(1)JC153/W32^P$) males have a weaker shaking phenotype than any of the V mutants suggesting that the shaking activity phenotype is not equivalent to the deficiency. Also note that the $B55^D$ element causes a dominant shaking phenotype by itself indicating that functions related to the membrane repolarization are encoded distal to *B55* and perturbed by this rearrangement.

that many double lethal heterozygous female combinations give rise to male escapers in a noticeable frequency. The escapers included representatives of both lethals whenever the flanking markers allowed their identification. These escapers lived for a short time (2 days maximum) with obvious movement impairments, including failure to mate normal females. Not all *trans*-heterozygotes give rise to viable escapers. The heterozygotes that yield escapers most frequently are HL/ME combinations. This effect is comparable to the result obtained in the germ line clones (see above) and leads to the same interpretation. It is plausible that the HL and ME products coparticipate in their normal functions by means of multimeric

structures. However, in this case the formation of hetero- rather than homomultimers is suggested

## DISCUSSION

The genetic analysis of the *Shaker* region indicates that mutations along 350 kb show either similarities in their neural phenotypes, antimorphic behavior in gene dosage tests or maternal rescue of lethality in *trans*-heterozygotes of certain combinations. Based on these features we refer to the interval between *JC153* distal and *S4010* as the *Shaker* gene complex.

A previous study of the *V* alleles in gene dosage experiments indicated that these mutations are antimorphs (TANOUYE, FERRÚS and FUJITA 1981). The present report on the characteristics of the ME region and the existence of lethals in this interval also indicates that the ME lethals are antimorphs. A further indication of antimorphism is obtained with the germ line clones of the ME lethal *1929* and the HL lethal *1614*. The electrophysiological phenotype of the *V* alleles, the tested ME and HL lethals and the *V7* breakpoint is another common trait.

Any proposition about the functional organization of the *ShC* must account for the antimorphic behavior of all the mutations analyzed. It should be realized that the gene dosage tests, in which the definition of antimorphs is based, do not provide information directly about the molecular consequences of the mutational events. The molecular basis of antimorphs can be either modifications of a gene product so that the novel entity perturbs the normal function, or the abolition of the mutated gene product. In this latter case, the absence of a product can behave as an antimorph if the normal function is accomplished by a multimer in which the product in question used to be included. We are pointing out that defects in product balance could, under some circumstances, meet the usual criteria for antimorphism.

We propose that both classes of antimorphs exist among the *ShC* mutants. The first class refers to those mutations that yield a modified gene product which, by itself or as part of a multimer, give rise to a new biological function. To this class belong the viable alleles and most EMS and ENU induced lethal mutations in particular *1929* and *1614* which yield viable lethals in germ line clones. The second class consists of those mutations which, we believe, yield reduced product and which are antimorphs because they alter the stoichiometry with respect to other components of the multimer.

The paradox between the lethal condition of ME mutants and the viability of $ME^-$ flies can be understood if the various functions coded in this region participate in a common pathway irrespective of the mechanism by which this is done (*e.g.*, multimers and feed back regulation). In certain metabolic pathways,

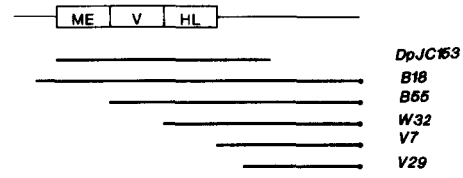



FIGURE 10.—Diagram of most frequently used rearrangements in the analysis of *ShC*.

the accumulation of intermediaries has a toxic effect while the abolition of the entire pathway is less deleterious due to alternative routes.

Within the context of this analysis, the meaning of multimer is somewhat different from the common use in biochemistry. We do not mean to imply a structure whose components are physically and permanently bound, rather we refer to the functional coupling between proteins that might assemble transiently. The required flexibility of biological systems yet maintaining an exquisite coordination, possibly relies in this kind of interaction. With the exception of some biochemical pathways, there are not many examples of clusters of functionally related genes in eukaryotes. We feel that this dearth of antecedents is only a consequence of the limitations for sophisticated genetic analysis in most organisms. Nonetheless, the structural elements of muscles have received considerable attention. Several muscle component genes are clustered in Drosophila (KARLIK *et al.* 1984), mouse (WEYDERT *et al.* 1985) and Caenorhabditis (LANDEL *et al.* 1984). Interestingly, mutations in this latter case belong to the antimorph class. In Drosophila, myofibril assembly is dependent on the proper stoichiometry of actin and myosin molecules. Trans-heterozygotes for the structural genes for these molecules show near normal muscle structure while heterozygotes of any of them separatedly have quite abnormal muscles (BEALL, SEPANSKI and FYRBERG 1989). This observation might be equivalent to ours on the maternal rescue of ME/HL lethal combinations. Evidences from the genetic analysis of nonclustered genes in Drosophila whose mutations affect muscle biology seem to indicate that they are functionally related (HOMYK and EMERSON 1988; DE LA POMPA, GARCIA and FERRÚS 1989). The structural genes for enzymes involved in certain pathways are frequently clustered. One such example in Drosophila is the cluster of 18 genes related to catecholamine metabolism (PENTZ, BLACK and WRIGHT 1986). No mechanistic interpretation can be offered still for any of these examples. However, it is likely that, if the genetic analysis becomes more elaborate and the studies more systematic, general trends on the functional organization of clustered and disperse gene families will emerge.

Our working hypothesis on the functional organization of the *Shaker* complex proposes that different transcriptional products from *ShC* give rise to combi-

**TABLE 6**

**Frequencies of escapers from *trans*-heterozygous combinations of Sh mutants**

| | *305* | *2215* | *1359* | *387* | *1929* | *459* | $Sh^{M}$ | $Sh^{KS133}$ | *2288* | *1614* | *484* | *3014* | *174* | + |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *305* | | | | | | | | | | | | | | |
| *2215* | | | | | | | | | | | | | | |
| *1359* | | | | | | | | | | | | | | |
| *387* | | | | | | | | | | | | | | |
| *1929* | | | | | | | | | | | | | | |
| *459* | 0.2 | | | | | | | | | | | | | |
| $Sh^{M}$ | | | | | | | | | | | | | | |
| $Sh^{KS133}$ | | | | | | | | | | | | | | |
| *2288* | | 1 | 1 | | | 0.2 | | | | | | | | |
| *1614* | 0.2 | 0.4 | 0.2 | 0.4 | | | | 7.4 | | | | | | |
| *484* | 15.4 | 8.8 | 7.8 | 8.6 | 1.2 | 1 | 3 | 0.4 | 0.2 | 1.2 | | | | |
| *3014* | 0.4 | 0.4 | 0.4 | 0.4 | 0.2 | | | | 1.4 | 0.4 | 6.6 | | | |
| *174* | | | | | | | | | 0.2 | | 1.2 | | | |
| + | | | | | | | | | | | 7 | | | 100 |

Numbers indicate the percentage of escapers among a minimum of 2000 offspring from crosses ♀ **1/*2* × ♂ *FM6*. These crosses were used also for the measurement of recombination. Recombinants were distinguished from escapers by the exchange of flanking markers and subsequent behavior of recovered males. Blank spaces indicate 0 escapers except for *V/V* combinations which were not measured.

nations of multimeric, possibly multifunctional, aggregates. In this context, seemingly different processes such as membrane repolarization, muscle structure and oogenesis could have a common genetic base.

The normal MEI, II and III products coded in the ME region seem to be relevant during oogenesis because the entire region, which contains lethal mutations in the three complementation groups, can be obtained as a viable deficiency only when the maternal oogenesis was performed by euploid cells. Indeed, the Northern analysis of this region has shown the abundant expression of ME transcripts in oocytes (BAUMANN *et al.* 1987). However, it is likely that the ME functions are not limited to oogenesis since the lethal phases of different mutants occur at various stages during development and mutant phenotypes can be observed in other tissues. Also, not all *ME* functions are equivalent. For instance, the *ME* $I^{+}$ function is required for cell viability while *ME* $II^{+}$ and $III^{+}$ are not. The lethality of all genotypes observed after germ line clones with the *ME II* mutant *1359*, suggests that the *ME* $II^{+}$ function(s) is essential during early embryogenesis and not rescuable by the zygotic genome. However, the pupal lethal phase of the mutant suggests further, that the *ME* $II^{+}$ function(s) is required also at this later time of postembryonic development. Interestingly, both stages have in common major changes of the neural pattern.

The dominant enhancing effect of *B27* upon $Sh^{KS133}$ and the dominant shaking phenotypes of the *ME III* lethal *459* and the $B55^{D}$ element, suggest a functional relationship between ME and V products. Possibly, some of the ME functions, in addition to their essential contributions to oogenesis, participate in some processes where $K^{+}$ currents are involved. Recent data from the molecular analysis of ME products indicate that a protein kinase is coded in this region (our unpublished results). Even though it is not yet known if this kinase actually uses the $K^{+}$ channels coded in the V region as a substrate, the V products contain suitable sequences to be targets of phosphorylation (PONGS *et al.* 1988).

The normal products coded in the V region are the best known to date at the molecular level (SCHWARTZ *et al.* 1988; PONGS *et al.* 1988; KAMB, TSENG-CRANK and TANOUYE 1988). It is proven that they act as $K^{+}$ channels of various kinds (IVERSON *et al.* 1988; TIMPE *et al.* 1988). The Western blots stained with antibodies against synthetic peptides show that $Sh^{M}$ and $Sh^{KS133}$ alleles yield protein(s) with the normal $M_r$ (BARBAS *et al.* 1989) supporting the interpretation of the V mutations as antimorphs due to modified gene products (TANOUYE, FERRÚS and FUJITA 1981). The *in situ* hybridization of exon specific riboprobes demonstrate that all V products are expressed in the nervous system and that most alleles do not perturb this expression (PONGS *et al.* 1988; our unpublished data). Finally, the transformation experiments with truncated cDNAs from the V region show that a normal fly can be transformed into a mutant (GISSELMAN *et al.* 1989).

Concerning the HL region, it is remarkable the high number of complementing lethals found in this relatively short interval (Figures 3 and 6). The expected dominant lethal mutations have been obtained only after X-ray mutagenesis resulting in chromosomal rearrangements. The categories of mutations in the HL interval range from viables to dominant lethals including semilethals, recessive lethals and semidominant lethals. The analysis of complementation (Fig. 6) indicates that a viable (*hdp*), a recessive lethal (*1614*) and a semidominant lethal (*8384*) belong to

the same complementation group. We take these observations as an indication that the constituents of the HL region might be functionally very similar (hence the common mutant phenotypes) and their functional genomic organization be subject to quantitatively different degrees of perturbation (hence the range of viabilities). The large number of complementation units in HL might be viewed as an indication of a wealth of transcription units, however, the ongoing studies at the molecular level show less transcription units than complementation groups (our unpublished data). In fact, the discrepancies between functional complementation and physical location of *174* and *1614* suggests the existence of functional links between these HL components. The observation that DL mutations have been obtained only among X-ray treated chromosomes and not among those treated with EMS, suggests that a perturbation of the physical organization of the DNA is required to yield such a phenotype. At present, we entertain two alternative working hypotheses on the functional organization of the HL region: a) the $HL^+$ function is represented by a regulatory, nontranslatable (hence not altered by point mutations) stretch of DNA, b) the haplolethality is the result of the combined depletion of a number of functionally related products. This combined depletion would be attained only after a polar effect from chromosomal rearrangements. If this latter hypothesis were correct, the large number of complementation units could be due to protein complementation (ZABIN and VILLAREJO 1975). Another haplolethal region of Drosophila's genome is located in 83 DE, albeit in this case it is also triplolethal. A mutational analysis of this region searching for dominant lethals has yielded also mostly chromosomal rearrangements rather than point mutations (ROEHRDANZ and LUCCHESI 1980). It is plausible that both cases (83 DE and 16 F) share a common genetic organization.

Aside from the $K^+$ current, the precise nature of the other functions encoded in *ShC* is still under investigation. The available evidence from the ongoing studies indicate that a protein kinase is encoded in the ME region and that a $Ca^{2+}$ binding protein and a calmodulin-like protein are encoded in the HL region (J. A. BARBAS *et al.* and I. KRAH-JENTGENS *et al.*, submitted for publication). Furthermore, all these products are expressed in the nervous system. Although these observations can not be taken as a demonstration of the hypothesis derived from the genetic analysis they are in strict agreement with it. At present, we can only say that most, if not all, *ShC* functions relate to the biology of $K^+$ channels since the abnormal CGF action potentials found in several mutants (*387*, *1614*, *174*, *V7*) are similar to those exhibited by the V mutations.

It is a fact that $K^+$ currents are the most diverse type of ionic currents (RUDY 1988; HILLE 1984). It is also a fact that certain $K^+$ currents are key processes in the modulation of synaptic efficacy (CROW 1988; BYRNE 1987; WALTERS and BYRNE 1983; KANDEL and SCHWARTZ 1982). To accomplish this role, a number of biochemical activities such as phosphorylation, dephosphorylation, nucleotide binding and translocation of cytoskeletal proteins must be tightly coupled with the $K^+$ channels (LOGOTHETIS *et al.* 1987; NORTH *et al.* 1987; ASHCROFT 1988; LEVITAN 1988). Furthermore it is likely that this functional coupling requires also a structural coupling in the form of a multimer. We hypothesize that the *ShC* encodes a large family of products which form multifunctional combinations in hetero- as well as homomultimers. Concerning the biology of $K^+$ currents, we propose that the functional coordination required among $K^+$ channels and their modulating factors might be a consequence of the genetic organization of the *ShC* (see also FERRÚS, LLAMAZARES and GAUNITZ 1988).

There is no reason to suspect that all modulating elements of $K^+$ channels will be clustered within *ShC*. We have isolated the gene *tetanic* (*tta*) as a possible *trans*-regulator of *ShC* expression using a gene dosage sensitivity test (ORGAD *et al.* 1989). Perhaps not as a surprise, *tta* mutations alter the levels of a specific protein phosphatase (ORGAD *et al.* 1989) which is a required modulating element for phosphorylated substrata.

The work with *Shaker* in Drosophila has facilitated the isolation of homologous $K^+$ channels in vertebrates (STUHMER *et al.* 1988; BAUMANN *et al.* 1988; TEMPEL, JAN and JAN 1988). Aside from the utilitarian aspect of this work, we feel that the study of this gene complex and its regulation will be informative about the functional organization of the genome as the unit with true biological significance. The genetic analysis performed so far provides information about the existence of relationships among the identified elements. However, we are proposing that a higher level of genomic organization exists whereby more elaborated features of the biology of $K^+$ channels are specified. Up to now we have identified the partners maintaining functional relationships. From now on we have to unravel the language that define this higher level of genetic organization.

This report is the account of ten years of work on this subject. It began at the California Institute of Technology, continued under very difficult conditions at the Centro de Biologia Molecular and finished at the Instituto Cajal. We wish to thank those colleagues who helped and encouraged us during these years in various ways, most notably: S. BENZER and J. MODOLELL. Also we are in debted to J. MERRIAM and G. LEFEVRE who provided essential chromosomal rearrangements. Our colleagues in the laboratories of Madrid and Bochum contributed with their criticisms. Funds were obtained

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