Coordinately and Differentially Mutable Activities of *torpedo*, the *Drosophila melanogaster* Homolog of the Vertebrate EGF Receptor Gene

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ABSTRACT

The *torpedo (top)* locus of Drosophila encodes the fruitfly homolog of the vertebrate epidermal growth factor receptor gene and the *neu* proto-oncogene. We have isolated 13 *top* alleles in a screen for mutations failing to complement the female sterility of top^1, a recessive maternal effect allele that disrupts the establishment of the dorsoventral pattern of the egg shell and embryo. Several alleles recovered in this screen are zygotic lethal mutations; genetic analysis of these alleles has demonstrated that *top* is allelic to the embryonic lethal locus *faint little ball*. The 13 mutations recovered in our screens and 19 previously isolated *top* alleles have been genetically characterized through complementation tests with a series of hypomorphic and amorphic alleles. Nearly every *top* allele fails to complement the maternal effect sterility of top^1. Complementation tests show that the gene is required not only for oogenesis and embryogenesis, but also for pupal viability, for the growth of certain imaginal discs and for the patterning of specific ectodermal derivatives of the imaginal discs. Complementation analysis further demonstrates that the *top* lesions can be divided into general phenotypic categories: alleles affecting all gene activities in a coordinate manner, alleles preferentially affecting embryogenesis, alleles preferentially retaining oogenesis activity and alleles differentially affecting the development of specific imaginal disc derivatives. Correlations observed between the various developmental defects produced by *top* lesions suggest that the gene possesses several differentially, though not independently, mutable activities.

RECEPTOR tyrosine kinases play a central role in the regulation of cell proliferation and differentiation in vertebrates. This class of proteins includes the insulin receptor, receptors for a number of peptide growth factors and several of the known oncogenes. Biochemical and molecular studies using avian and mammalian cell lines have provided a wealth of information on the structure and function of these proteins. All receptor tyrosine kinases are structurally similar, consisting of an extracellular ligand binding domain, a hydrophobic membrane spanning region and an intracellular tyrosine kinase domain (reviewed in YARDEN and ULLRICH 1988). Although tissue culture studies have provided insights into the role these receptors play in the proliferation and differentiation of specific cell types, they have so far not proven useful for investigating the function of these genes in the development of the organism as a whole.

The molecular identification of Drosophila genes homologous to vertebrate receptor tyrosine kinases (reviewed in SHILO 1987) suggested that the fly can serve as a system for the genetic and developmental analysis of such transmembrane receptors. DER, the first receptor tyrosine kinase identified in Drosophila, was detected by LIVNEH *et al.* (1985) and WADSWORTH, VINCENT and BILODAEU-WENTWORTH (1985) by virtue of its homology to v-*erb-B*, an avian oncogene derived from the epidermal growth factor receptor (DOWNWARD *et al.* 1984). Determination of the complete amino acid sequence of DER indicated that it is equally related to the EGF receptor gene and neu proto-oncogene (SCHECHTER *et al.* 1984; ULLRICH *et al.* 1984; SCHEJTER *et al.* 1986; SCHEJTER and SHILO 1989), while molecular studies have shown that the gene is expressed throughout the life of the fly in a variety of tissues (LEV, SHILO and KIMCHIE 1985; KAMMERMEYER and WADSWORTH 1987). PRICE, CLIFFORD and SCHÜPBACH (1989) and SCHEJTER and SHILO (1989) have recently demonstrated that DER is encoded by the *torpedo* (*top*) locus. In the following work, we describe a genetic characterization of *top* intended to lay the foundation for a comprehensive analysis of the developmental role of this receptor in the fly.

The original *top* lesion, top^1, is a maternal effect lethal mutation isolated in a screen for recessive female sterile loci on the second chromosome of Drosophila. Females homozygous for this mutation produce eggs with ventralized egg shells that give rise to correspondingly ventralized embryos; these pattern alterations result from shifts in cell fate rather than localized cell death (SCHÜPBACH 1987). In Drosophila

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the egg shell is produced by the mesodermally derived follicle cells, while the establishment of the embryonic body plan is regulated by asymmetrically distributed determinants within the oocyte (a cell of germline origin). Since a mutation in *top* alters both patterns the gene must participate in a process by which the spatial pattern of the germline and somatic components of the egg chamber are coordinately established or maintained. *top* is unusual among the known female sterile loci affecting both the chorion and embryonic patterns, as *top* is required exclusively in the somatic tissues of the female (WIESCHAUS, MARSH and GEHRING 1978; WIESCHAUS 1979; FREY and GUTZEIT 1986; SCHÜPBACH 1987; MANSEAU and SCHÜPBACH 1989). Given the homology in predicted protein sequence, this suggests that the *top* product acts in the follicle cells as the receptor for an intercellular signal required for the establishment of polarity in the developing egg chamber.

This report presents a genetic demonstration that the *top* locus corresponds to the zygotic embryonic lethal gene *faint little ball,* first identified by NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING (1984). We further show that *top* is required not only for embryogenesis and oogenesis, but also for a variety of other developmental processes occurring throughout the life of the fly. Lesions in the gene lead to pupal lethality, disrupt the growth of certain imaginal discs and produce discrete pattern defects in the adult derivatives of the eye-antennal, humeral, wing, leg and genital imaginal discs. A phenotypic analysis of this gene indicates that several of these developmental processes can be preferentially, though not exclusively, affected by particular *top* lesions.

MATERIALS AND METHODS

Stocks: The mutations, chromosomal rearrangements and balancer chromosomes used in this study are described in LINDSLEY and GRELL (1968) or LINDSLEY and ZIMM (1985) unless otherwise noted.

CyO DTS-100 and *CyO DTS-513* are *CyO*-derived balancer chromosomes bearing dominant temperature sensitive lethal mutations (Falke and Wright 1973). The lethal phase of *CyO DTS-100* begins after embryogenesis and continues throughout the life cycle, while the temperature sensitive period of *CyO DTS-513* is restricted to embryogenesis (STEWARD and NÜSSLEIN-VOLHARD 1986).

Fs(2)1 is a dominant second chromosomal female sterile mutation that does not affect male fertility. Females heterozygous for *Fs(2)1* produce spindle-shaped eggs that never hatch (SZABAD, ERDÉLYI and SZIDONYA 1987). top^1, $gurken^{HK}$ and $gurken^{WG}$ are described in SCHÜPBACH (1987); $cappuccino^{RK}$ and $spire^{RP}$ are described in MANSEAU and SCHÜPBACH (1989). *25.11* is a multiply marked tester chromosome bearing the female sterile mutations $cappuccino^{RK}$, $gurken^{WG}$, $chalice^{WP46}$, $spire^{RP}$ and $torpedo^1$, as well as the eye color mutations *cn* and *bw*.

faint little ball alleles (NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984; O'DONNELL *et al.* 1989) were kindly provided by R. BOSWELL, C. NÜSSLEIN-VOLHARD, J. O'DONNELL and E. WIESCHAUS.

Fly food: Flies were grown on cornmeal agar medium described in CLINE (1978). Apple juice agar medium used for egg collections is described in WIESCHAUS and NÜSSLEIN-VOLHARD (1986).

EMS mutagenesis: To generate new *top* alleles, males homozygous for a lethal-free, previously isogenized, second chromosome marked with *b pr cn sca* were fed 50 mM EMS in 1% sucrose essentially as described by LEWIS and BACHER (1968). The males were starved on wet paper towels for 4 hr, then transferred to bottles containing five Kimwipes soaked with 12 ml of a 1% sucrose, 50 mM EMS solution. After 24 hr, mutagenized males were transferred to fresh medium, allowed to recover for 24 hr, then mated to *b Tft/CyO DTS-100* virgin females (~50 males and ~150 females per bottle) at 18° as shown in Figure 1.

F_1 *(b pr cn sca)* /CyO DTS-100* virgin females were mass mated to *25.11 /Fs(2)1* males at 18° for 3 days (Figure 1), then individually transferred to prewarmed vials at 29°. After 7 days, the females were discarded and the vials were maintained at 29°. Since *CyO DTS-100* acts as a dominant lethal at temperatures above 25°, all F_2 animals were either *(b pr cn sca)*/25.11* or *(b pr cn sca)*/Fs(2)1*; as *Fs(2)1* is a dominant female sterile mutation, the fertility of the *(b pr cn sca)*/25.11* females could be assayed by examining vials for the presence of first instar larvae 7–10 days after the eclosion of the F_2 generation. The absence of F_3 animals indicated either sterility of the F_2 parents or, alternatively, the presence of a lesion on the mutagenized chromosome that is lethal when in *trans* to the *25.11* chromosome; such lethal mutations could then be recovered through the F_2 *(b pr cn sca)*Fs(2)1* males. Putative *top* alleles were retested by crossing *(b pr cn sca)*/25.11* males to top^1 *bw/CyO* and $gurken^{WG}$ *cn bw/CyO* virgin females. The resulting *(b pr cn sca)*/*top^1 *bw* and *(b pr cn sca)*/*$gurken^{WG}$ *cn bw* females were tested for fertility at 18° and 29°, and stocks were established by mating *(b pr cn sca)*/CyO* males and virgin females *inter se.* Of 5222 mutagenized chromosomes screened in this manner, 9 carried *top* lesions.

An additional 3250 chromosomes were examined in a similar screen employing the *Fs(2)D* dominant female sterile mutation. In this screen, however, single mutagenized chromosomes were obtained from both F_1 males and females. A total of 1197 *(b pr cn sca)*/CyO DTS-100* F_1 virgin females were mated to *25.11/Fs(2)D* males, and their progeny were screened for sterility as described above. In addition, 2053 *(b pr cn sca)*/CyO DTS-100* F_1 males were transferred to separate vials containing two *25.11/CyO DTS-100* females. The F_1 animals were allowed to mate in the vials for 1 week at 29°, then discarded. The F_2 generation, consisting solely of *(b pr cn sca)*/25.11* animals, was examined for fertility. This screen yielded 2 *top* mutations.

Two additional *top* alleles were isolated in a pilot screen of 267 *b pr cn sca* chromosomes mutagenized with 50 mM EMS and examined over a $gurken^{HK}$ *cn* top^1 *bw* tester chromosome. This mutagenesis screen involved mating individual F_1 *(b pr cn sIa)*/CyO DTS-513* males and virgin females to $gurken^{HK}$ *cn top*1 *bw/CyO DTS-513* virgins or males in single vials at 29°. Because of the high frequency of *CyO DTS-513* escapers in this screen the F_2 *(b pr cn sca)*/*$gurken^{HK}$ *cn* top^1 *bw* females were hand selected and tested for sterility in egg laying blocks as described in WIESCHAUS and NÜSSLEIN-VOLHARD (1986).

Complementation tests and determination of lethal phenotypes: Adult phenotypes of *top* heteroallelic combinations were determined in the following manner. Three to five top^x/CyO or $top^x/SM1$ virgin females were crossed to an equal

number of top^y /CyO or top^y/SM1 males at 25°. Fifty top^x/top^y progeny (unless otherwise noted) from each cross were scored for wing, eye and thoracic bristle defects at 25× under a Zeiss dissecting microscope, then placed in 70% ethanol for storage. To score cephalic structures, heads from an equal number of male and female flies of each genotype were dissected in 70% ethanol, bleached in 10% NaOH at 60° for 15–30 min, gently squeezed between the tips of a forceps to remove eye pigments, then washed in distilled H_2O at 60° for at least 30 min. Bleached heads were mounted in a drop of Hoyer's mountant (WIESCHAUS and NÜSSLEIN-VOLHARD 1986) on a microscope slide, allowed to clear overnight at 40°, then flattened with weights. Head bristle, arista and ocellar defects were scored under a Zeiss compound microscope at 100×. The severity of compound eye defects were initially determined by examining the heads of 50 flies under a dissecting microscope, then verified by examining ten mounted heads at 400× using phase contrast optics. To avoid bias resulting from possible sex-specific differences in eye phenotypes, an equal number of male and female flies were examined whenever practicable. To examine the female sterile phenotype of the various *top* mutations, *top* females were transferred to egg laying blocks as described in WIESCHAUS and NÜSSLEIN-VOLHARD (1986). Eggs were examined at 25× under a Zeiss dissecting microscope.

To determine whether the phenotypes observed in the complementation tests are reproducible, the top^1 /top^1, top^1 /top^{CO}, and top^1/top^{EB} complementation tests were repeated under the same conditions as the original complementation tests. In most cases, the frequency of bristle and wing vein defects observed in the repeat crosses differed by no more than 10% from the frequencies seen in the first round of tests, and the roughened eye and female sterile phenotypes also exhibited little variability.

To verify that the phenotypes seen over top^1 accurately reflect each mutation's eye, wing, bristle and oogenesis activity rather than particular properties of or specific allelic interactions with the top^1 lesion, complementation tests were also performed between all *top* alleles and top^{CJ}, top^{EE38} and top^{CA}. In general the mutant phenotypes correlated well with the severity of the tester allele used. The inconsistent behavior of some adult structures (*e.g.*, the anterior crossvein) seen in comparisons between heteroallelic combinations employing different adult viable alleles (Figures 9 and 10) may reflect either region-specific variability in the expression of the mutant phenotype or allele-specific interactions between different mutations. Such variability, however, is exhibited only by a few structures and is rarely of great magnitude. In nearly every case, the regional specificities of each *top* allele's adult defects and the severity of its defects relative to those of other *top* alleles seen in complementation tests with top^{CJ}, top^{EE38} and top^{CA} are in accord with those observed in combination with top^1.

To limit phenotypic variation due to genetic background, adult phenotypes of the *top* mutations were assayed, whenever possible, in combination with an adult viable allele induced in a different genetic background. The genetic background of top^1 differs from that of the other lesions examined in this study; thus all heteroallelic combinations involving top^1 satisfy the above condition. Most complementation tests involving top^{CJ}, top^{EE38} and top^{CA} also meet this condition. When mutations derived from the same genetic background as top^{CJ}, top^{EE38} or top^{CA} were examined over these adult viable alleles, a recombinant version of the chromosome carrying the adult viable tester allele was used for the complementation test. Since many of the *top* alleles examined in this study have not been extensively outcrossed, it is possible that some of the phenotypes are modified due to the effects of background mutations.

Embryonic cuticles were prepared as is described in WIESCHAUS and NÜSSLEIN-VOLHARD (1986) and examined under a Zeiss compound microscope at 63×, 160×, 250× and 400× with phase contrast optics. Chorions were prepared for photography by standard methods (WIESCHAUS and NÜSSLEIN-VOLHARD 1986). Scanning electron micrographs of compound eyes and ocelli were made using live flies; animals were examined with a JEOL JSM 840-II scanning electron microscope at 3–5 kV.

Determination of the *torpedo* postembryonic lethal phases: The following scheme was employed to determine the lethal periods of postembryonic lethal *top* allelic combinations. $Df(2R)top^{3F18}$/CyO virgin females were mated to top^x/Bc males (where top^x represents the semiviable allele top^{CA} or the pupal lethal allele top^{EB} or top^{EC20}. No cross produced significant numbers of dead embryos. The larvae from each cross were divided into two groups, those possessing darkly pigmented cells (Bc/$Df(2R)top^{3F18}$ and Bc/CyO) and those showing wild-type pigmentation (top^x/$Df(2R)top^{3F18}$ and top^x/CyO). The lethal phase of each combination was determined from the number of larvae, pupae and eclosing adults in the latter group. Although each mating yielded approximately equal numbers of *Bc* and Bc^+ pupae, only half of the Bc^+ animals reached adulthood. All eclosing non-*Bc* animals were genotypically top^x/CyO.

Determination of imaginal disc phenotypes: To determine whether *top* mutants dying early in pupation show imaginal disc abnormalities at earlier stages of development, animals of a representative pupal lethal genotype, top^{EC20}/$Df(2R)top^{3F18}$, were examined. *cn* $Df(2R)top^{3F18}$ *bw sp*/CyO females were crossed to *cn* top^{EC20} *bw*/CyO males. The adults were transferred to fresh vials daily. F_1 larvae were allowed to develop until they began crawling up the sides of the vials. top^{EC20}/$Df(2R)top^{3F18}$ larvae (which are homozygous for *cn* and *bw* and possess unpigmented Malpighian tubules) could be distinguished from their genetically wild-type sibs (possessing yellow Malpighian tubules). Imaginal discs of mutant and wild-type larvae were dissected out in a drop of distilled H_2O, fixed in 2.5% glutaraldehyde in phosphate-buffered saline for approximately 30 min, then mounted on microscope slides in a drop of distilled H_2O and examined under the compound microscope.

RESULTS

Isolation of *torpedo* mutations

At the outset of this study, only one *torpedo* mutation, top^1, had been recovered. This lesion is homozygous viable and female sterile (SCHÜPBACH 1987). To identify new alleles of *top*, an ethyl methanesulfonate (EMS) mutagenesis was performed. Mutagenized chromosomes were tested for female sterility or lethality in *trans* to a tester chromosome carrying top^1. Thirteen *top* alleles were recovered from 8739 mutagenized chromosomes examined in our screens (see MATERIALS and METHODS and Figure 1). All 13 alleles are recessive, all are viable in combination with top^1 and none is conditionally female sterile.

An examination of *top* alleles recovered in the EMS screens showed that seven of these 13 mutations, both in the homozygous state and in *trans* to $Df(2R)top^{3F18}$ are required zygotically during embryogenesis and

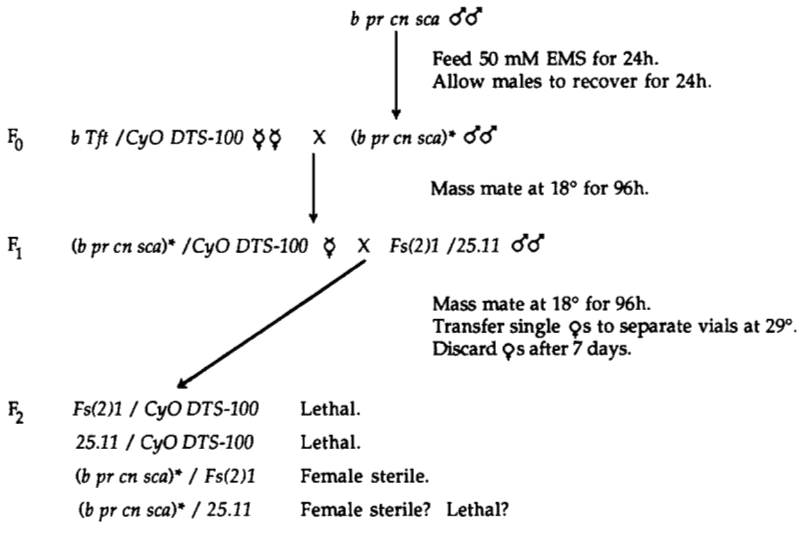

FIGURE 1.—Mutagenesis screen for the isolation of *torpedo* mutations. *CyO DTS-100* is a balancer chromosome that carries a dominant temperature-sensitive mutation that is lethal at 29°; *Fs(2)1* is a dominant female sterile mutation that has no effect on male fertility; *25.11* is a tester chromosome carrying the top^1 lesion. Single mutagenized chromosomes were recovered and balanced over the *CyO DTS-100* chromosome in F_1 females. Individual females were mated to *Fs(2)1/25.11* males to establish lines of single mutagenized chromosomes. Progeny of these matings were raised at the restrictive temperature to kill flies carrying the balancer; consequently, the surviving flies were of either the *(b pr cn sca)*/Fs(2)1* (female sterile) or *(b pr cn sca)*/25.11* genotype. Vials containing F_2 lines derived from mutagenized chromosomes that are female sterile or lethal in combination with the *25.11* tester could be identified by the absence of F_3 progeny. Chromosomes carrying putative *top* mutations were recovered through *(b pr cn sca)*/25.11* or *(b pr cn sca)*/Fs(2)1* males and retested over top^1.

exhibit lethal phenotypes identical to those produced by lesions in the zygotic embryonic lethal locus *faint little ball* (*flb*, 2–101; NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING 1984) (Table 1). To demonstrate that *top* and *flb* mutations are allelic, 14 EMS-generated *flb* alleles isolated by NÜSSLEIN-VOLHARD, WIESCHAUS and KLUDING (1984), as well as a single ethylnitrosourea-induced and three EMS-induced *flb* mutations isolated by O'DONNELL *et al.* (1989) (Table 1) were crossed to top^1 and top^{CJ} and scored for their ability to complement the oogenesis defects. All *flb* alleles fail to complement the two viable *top* mutations for female sterility (see below). By the same token, top^{CO}, isolated as a female sterile lesion over top^1, fails to complement the zygotic lethality of any *flb* mutation (data not shown). Therefore *top* and *flb* alleles are lesions in the same gene.

Stage and spatial specificity of the *torpedo* phenotypes

To determine the temporal and tissue requirements for the *top* gene product, the 32 available *top* mutations were phenotypically examined in the homozygous state and in combination with selected alleles. This analysis revealed that lesions at the top locus produce four developmentally distinct defects: zygotic embryonic lethality, zygotic pupal lethality, imaginal disc pattern abnormalities and a maternal effect ventralization of the egg shell and embryo. Characterization of the mutant phenotypes has provided insights into the role *top* plays in development, and the correlations observed between the various defects have shed light on the functional organization of the gene. The phenotypes upon which these developmental and functional analyses are based are described below.

***torpedo* is required zygotically for embryonic development:** Twenty-five of the 32 available *top* lesions exhibit embryonic lethality (Table 1). These mutations show a range of "faint little ball" ("flb") lethal phenotypes that can be clearly arranged in order of severity: 14 lesions show a severe "flb" lethal phenotype, five a moderate "flb" phenotype and six exhibit a weak embryonic lethal phenotype. Animals homozygous for a deficiency of the locus or a strong *top* point mutation die as embryos that are tightly curled and folded back upon themselves, show anterior and posterior cuticular holes and possess few, if any, ventral setae (Figure 2D). Embryos of moderate phenotypes (Figure 2C) also show a large anterior hole, produce a poorly differentiated head skeleton and are curled upon themselves. These animals, unlike severe mutant embryos, often undergo partial germband retraction and possess nearly intact posterior cuticle. Weak embryonic lethal alleles (Figure 2B) produce an essentially intact cuticle and possess well-defined denticle belts. Mutant denticle bands are, however, composed of fewer and smaller ventral setae than wild-type denticle belts. Weak "flb" animals also exhibit reductions in the ventral plate and H-piece of the head skeleton and often show a "U-shaped" or "tail up" phenotype.

***torpedo* is required for pupal viability:** Three *top* alleles allow homozygous embryos to develop normally but always cause lethality prior to eclosion of the adult (Table 1). To avoid possible obscuration of the terminal phenotypes of these postembryonic lethal alleles by background mutations, we determined their lethal phases over $Df(2R)top^{3F18}$. In combination with a deletion of the locus, two of these mutations die during early pupation. Animals homozygous for a fourth allele, top^{CA}, usually die during postembryonic development; the few top^{CA} homozygotes that do eclose survive poorly as adults. In *trans* to the deficiency, top^{CA}, too, exhibits pupal lethality (Table 1, see below).

TABLE 1

***torpedo* alleles used in this study**

Allele	Origin	Homozygous phenotype	Phenotype over *Df*	Reference[a]
top^{1}	EMS	Viable	Viable	1
top^{38}	EMS	Severe *flb*	Severe *flb*	2
top^{101}	EMS	Weak *flb*	Weak *flb*	2
top^{CA}	EMS	Semiviable	Late pupal	2
top^{CJ}	EMS	Viable	Viable	2
top^{CO}	EMS	Severe *flb*	Severe *flb*	2
top^{EA}	EMS	Postembryonic	Weak *flb*	2
top^{EB}	EMS	Postembryonic	Early pupal	2
top^{EC20}	EMS	Postembryonic	Early pupal	2
top^{ED16}	EMS	Late embryonic	Late embryonic	2
top^{ED26}	EMS	Intermediate *flb*	Severe *flb*	2
top^{EE38}	EMS	Viable	Viable	2
top^{EE39}	EMS	Intermediate *flb*	Intermediate *flb*	2
top^{EE42}	EMS	Severe *flb*	Severe *flb*	2
top^{1F26kb}	EMS	Weak *flb*	Weak *flb*	3
top^{1K35}	EMS	Severe *flb*	Severe *flb*	3
top^{1P02}	EMS	Severe *flb*	Severe *flb*	3
top^{2C82}	EMS	Intermediate *flb*	Intermediate *flb*	3
top^{2E07}	EMS	Weak *flb*	Intermediate *flb*	3
top^{2G31}	EMS	Intermediate *flb*	Intermediate *flb*	3
top^{2L65}	EMS	Severe *flb*	Severe *flb*	3
top^{2W74b}	EMS	Weak *flb*	Weak *flb*	3
top^{2X51}	EMS	Severe *flb*	Severe *flb*	3
top^{3B41}	EMS	Severe *flb*	Severe *flb*	3
top^{3B92}	EMS	Severe *flb*	Severe *flb*	3
top^{3C81}	EMS	Severe *flb*	Severe *flb*	3
top^{3C87}	EMS	Severe *flb*	Severe *flb*	3
top^{3F18c}	EMS	Severe *flb*	Severe *flb*	3
top^{JE1d}	EMS	Severe *flb*	Severe *flb*	4
top^{JE13e}	EMS	Severe *flb*	Severe *flb*	4
top^{JE14f}	EMS	Intermediate *flb*	Not determined	4
$top^{SH2b,g}$	ENU	Weak *flb*	Intermediate *flb*	4

[a] 1, Schüpbach (1987); 2, This work; 3, NÜSSLEIN-VOLHARD, WIESCHAUS AND KLUDING (1984); 4, O'DONNELL *et al.* (1989).

[b] These alleles are temperature-sensitive, exhibiting a more extreme "flb" phenotype at 29° than at 18°. No allele is, however, wildtype at 18°.

[c] This allele is a deficiency uncovering the 57D8,9-E1; 57F5-10 cytological interval (PRICE, CLIFFORD and SCHUPBACH 1989).

[d] Designated *l(2)57DEFa-1* in O'Donnell *et al.* (1989).

[e] Designated *l(2)57DEFa-2* in O'Donnell *et al.* (1989).

[f] Designated *l(2)57DEFa-3* in O'Donnell *et al.* (1989).

[g] Designated *l(2)57DEFa-4* in O'Donnell *et al.* (1989).

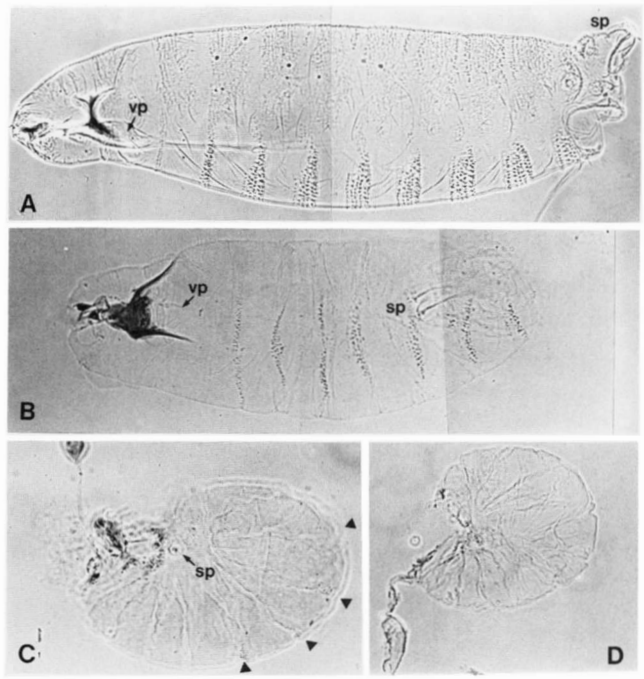

FIGURE 2.—Cuticular phenotypes of zygotic embryonic lethal *torpedo* alleles. (A) wild-type embryo. Anterior is to the left and dorsal is up. The ventral abdominal cuticle of the wild-type embryo is marked by eight prominent denticle belts, while the lateral and dorsal cuticle is covered by the dorsal hairs. The cephalopharyngeal skeleton, containing the ventral plate (vp), is clearly visible at the anterior of the embryo and the spiracles (sp) lie at the posterior end of the embryo. (B) top^{101}/top^{101} embryo: weak "faint little ball" phenotype. Mutant denticle bands are composed of fewer and smaller ventral setae than are wild-type denticle bands. The ventral plate (vp) of the top^{101} homozygote is reduced in size. The spiracles (sp) of the mutant point anteriorly, as the animal has not fully completed germband retraction. (C) top^{2C82}/top^{2C82} embryo: moderate "faint little ball" phenotype. Only four denticle bands (arrowheads) are visible in the mutant, and cephalic structures are severely reduced. Germband retraction has not occurred in this animal; a rudimentary spiracle (sp) lies just behind the remnants of the head skeleton. As is evidenced by its small size, the top^{2C82} embryo produces less cuticle than a wild-type embryo. (D) top^{CO}/top^{CO} embryo: severe "faint little ball" phenotype. This animal appears to lack all denticles and most head and thoracic cuticle. Germband retraction has not occurred. The severe mutant produces even less cuticle than the top^{2C82}/top^{2C82} animal.

***torpedo* is required for the development of several imaginal discs:** The role of *top* for pupal viability suggested that the gene may be needed for imaginal disc development. To investigate this possibility, imaginal discs from mature third instar larvae of the pupal lethal genotype $top^{EC20}/Df(2R)top^{3F18}$ were examined. We have observed abnormalities in three imaginal discs in these animals. In $top^{EC20}/Df(2R)top^{3F18}$ larvae, the portion of the eye-antennal disc that gives rise to the eye is reduced in size; on the other hand, the anterior lobe of the eye-antennal disc, which develops into the antenna, appears normal (Figure 3G). The development of the wing and haltere imaginal discs is also clearly altered in the mutant. Both discs are severely reduced in size in $top^{EC20}/Df(2R)top^{3F18}$ animals (Figure 3, E and F). In marked contrast to the eye-antennal, wing and haltere imaginal discs, the precursors of the prothoracic, mesothoracic and metathoracic legs appear to be morphologically normal in the mutant (Figure 3, E and F) (data not shown). We have not analyzed the labial, humeral or genital imaginal discs in the mutant.

Defects seen in mutant eye-antennal, wing and haltere imaginal discs indicate that *top* is necessary for the proliferation or maintenance of cells within these structures. The preferential size reduction of the eye portion of the eye-antennal disc demonstrates that a disc may show regional differences in its requirement for the *top* gene product.

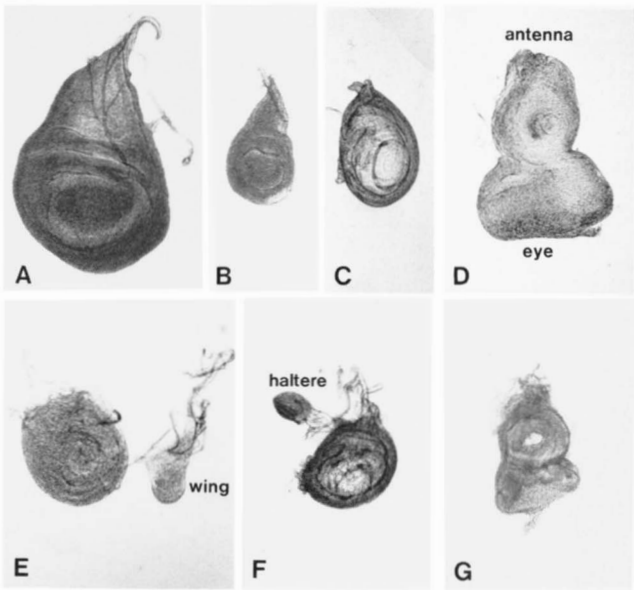

FIGURE 3.—*torpedo* is required for the growth of certain imaginal discs. Bright field micrographs of representative imaginal discs dissected from mature wild-type and $top^{EC20}/Df(2R)top^{3F18}$ third instar larvae. (A) Wild-type wing disc. (B) Wild-type haltere disc. (C) Wild-type metathoracic leg disc. (D) Wild-type eye-antennal disc. The portion of the eye-antennal disc giving rise to the compound eye and the portion developing into the antenna are indicated. (E) $top^{EC20}/Df(2R)top^{3F18}$ wing and metathoracic leg discs. (F) $top^{EC20}/Df(2R)top^{3F18}$ haltere and metathoracic leg discs. Although the mutant wing and haltere discs are severely reduced in size, $top^{EC20}/Df(2R)top^{3F18}$ metathoracic leg discs appear to be of normal size and morphology. (G) $top^{EC20}/Df(2R)top^{3F18}$ eye-antennal imaginal disc. The growth of the eye-antennal disc is nonuniformly disrupted in the mutant: the antennal half of the disc is nearly normal, while the region of the disc which develops into the compound eye is clearly reduced in size.

***torpedo* is required for the patterning of imaginal disc derivatives:** The remaining *top* alleles are homozygous viable and survive to adulthood over a deficiency of the locus (Table 1). The adult viable *top* mutations, when homozygous or in *trans* to one another, exhibit adult cuticular pattern defects and female sterility. The severity of these defects are greatly intensified when the alleles are placed over a deficiency. Structures affected in mutant adults include the compound and simple eyes, certain wing veins, specific cephalic and thoracic macrochaetae, the aristae, the claws, the sex comb of the male and the female analia. The various pattern abnormalities are described below.

torpedo mutations disrupt compound eye and ocellus development: Mutations in *top* disrupt the development of the compound eye. In *top* mutants, the facets of the compound eye are often irregularly shaped and sometimes fused, while the interommatidial bristles are abnormally distributed (Figure 4B). These defects give the eye a rough appearance. In addition to a disorganization of the ommatidia and their associated bristles, severely affected eyes also exhibit prominent dark spots along their anterior margin (Figure 4B). Scanning electron micrographs of mutant eyes show that many facets located in the anterior portion of the eye possess defective corneas: whereas the cornea of a wild-type facet is convex, the cornea overlying these mutant ommatidia is concave (Figure 4D). Since the corneal material at the center of the ommatidium is produced by the cone cells (PERRY 1968), a concave cornea may result from a defect in cone cell development (R. CAGAN, personal communication).

Mutant animals also show defects in the ocelli, or simple eyes. Severely mutant animals may completely lack the ocelli. In these animals, a shallow depression is found in the place of the simple eye, suggesting that the ocellar precursor cells either have died or have failed to undergo differentiation. Less severe mutants possess ocelli that are reduced in size (Figure 4F).

torpedo is necessary for proper wing venation: Another adult structure whose differentiation is reproducibly altered in *top* mutants is the wing. The shape and size of the wing appears to be normal, but two veins, the anterior crossvein (acv) and fourth longitudinal vein (L4), are defective. Whereas the anterior crossvein is often completely removed, the fourth longitudinal vein is never completely absent in the *top* genotypes we have examined. Reductions in gene activity produce gaps of varying size in the portion of L4 extending from a point slightly proximal to the acv to a point lying between the posterior crossvein (pcv) and wing margin (Figure 5B).

torpedo mutations alter the distribution of macrochaetae: The adult cuticle of *Drosophila melanogaster* possesses a number of precisely located large sensory bristles, the macrochaetae (Figure 6A). The majority of bristles affected in *top* mutants are severely reduced in size or deleted. The macrochaetae most sensitive to elimination are the ocellar (OC), posterior supraalar (pSA), anterior supraalar (aSA), posterior postalar (pPA), dorsal humeral (dH) and ventral humeral (vH) bristles. Other bristles are absent only in some mutant animals (Figure 6A). In contrast to the other macrochaetae affected by *top* mutations, the anterior postalar (aPA) and postvertical (PV) bristles are duplicated rather than removed. The degree of duplication ranges from a bifurcation of the bristle shaft to the formation of a complete ectopic bristle shaft and socket (Figure 6, C–E).

torpedo is required for the development of the arista: Adults of certain heteroallelic *top* genotypes exhibit reduced or absent aristae. The arista, the fourth and distalmost segment of the antenna, is derived from the center of the anterior portion of the eye-antennal imaginal disc (BRYANT 1978).

torpedo lesions affect the development of the leg: Mutant adults also exhibit pattern defects in the leg. Male flies of several *top* genotypes show abnormalities in the

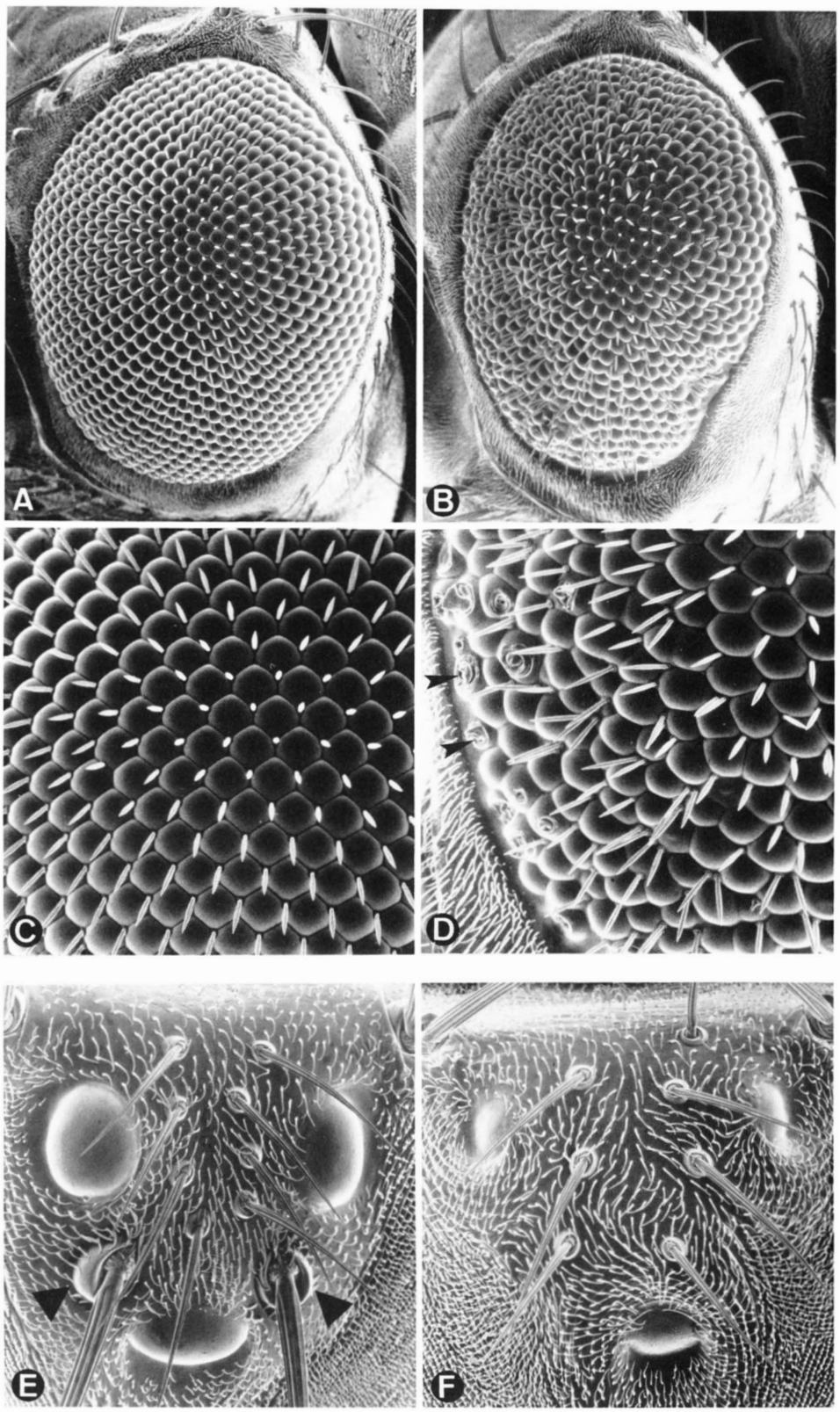

FIGURE 4.—*torpedo* is needed for the development of the compound eye and ocellus. (A) Scanning electron micrograph of a wild-type compound eye. Anterior is to the left. (B) Compound eye of a $top^{1}/Df(2R)top^{18A}$ heterozygote; $Df(2R)top^{18A}$ is a single-band deficiency removing the *top* locus (PRICE, CLIFFORD and SCHÜPBACH 1989). The facets of the mutant eye are of irregular shape and do not lie in straight rows. The interommatidial bristles are frequently clustered. (C) Detail of a wild-type compound eye. (D) Detail of the anterior margin of a $top^{1}/Df(2R)top^{18A}$ compound eye. Note that many facets along the edge of the eye exhibit corneal abnormalities (arrowheads). (E) Scanning electron micrograph of wild-type ocelli (simple eyes). Posterior is up. The ocellar bristles are marked by arrowheads. (F) $top^{1}/Df(2R)top^{3F18}$ ocelli. The mutant ocelli are reduced in size. Note also that the ocellar bristles are completely eliminated.

pattern of the sex comb. The sex comb, a male-specific structure located on the first tarsal segment of the foreleg, consists of a single anterior to posterior row of approximately 11 comb teeth. Mutant sex combs possess, on average, one additional tooth. Additionally, one or more teeth of *top* sex combs are displaced medially. A defect seen less frequently in the legs of *top* animals is the elimination of the tarsal claws, the distalmost leg structure; this abnormality occurs in flies of both sexes.

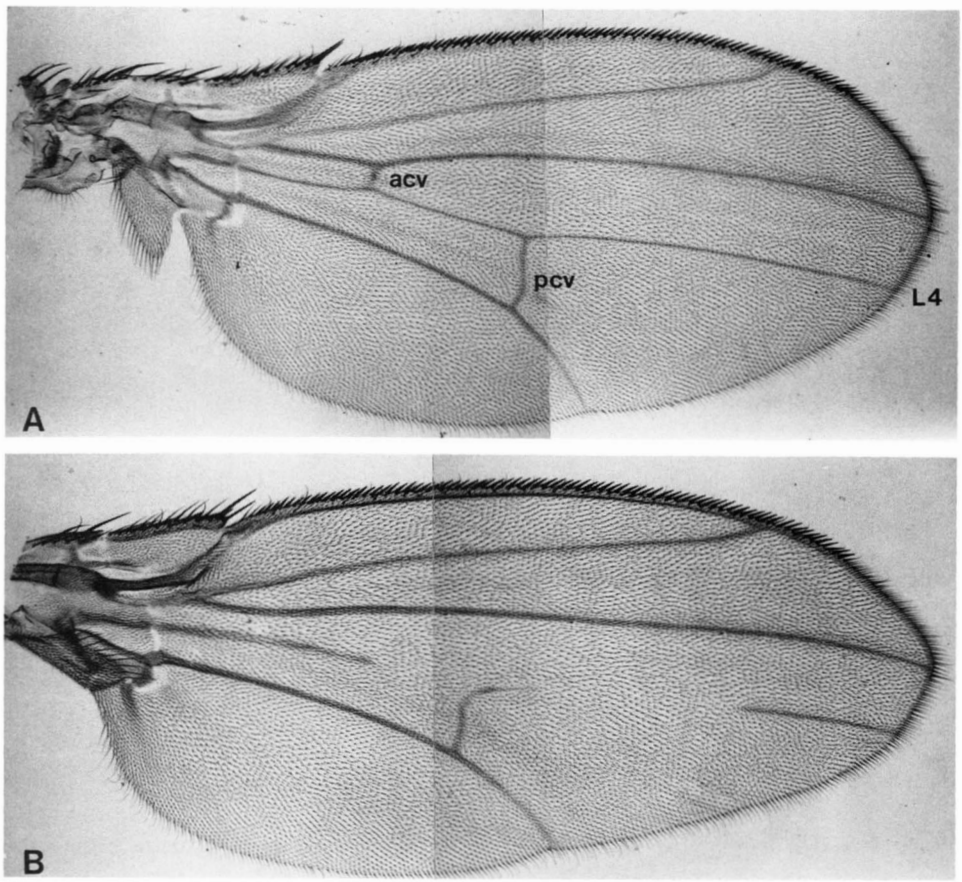

FIGURE 5.—*torpedo* mutations disrupt the development of specific wing veins. (A) Bright-field photomicrograph of a wild-type wing. The anterior crossvein (acv), posterior crossvein (pcv) and fourth longitudinal vein (L4) are marked. (B) top^1/top^{EB} wing. The entire anterior crossvein and the central portion of the fourth longitudinal vein are absent.

torpedo is needed for the development of the female genital disc: Severely mutant *top* females show defects in the anal plate, a derivative of the genital disc. The dorsal and ventral anal plates of affected females possess fewer bristles than wild-type analia and are correspondingly reduced in size. While the dorsal and ventral anal plates of a wild-type female contain roughly 20 and 18 bristles, respectively, the dorsal anal plate of a severely affected *top* female may entirely lack bristles and the ventral plate may possess as few as two bristles.

***torpedo* is required for oogenesis:** All *top* alleles fail to complement the female sterility of top^1 or top^{CJ}. Oogenesis defects of the heteroallelic combinations range from less severe to more severe than the top^1 phenotype. As is described in SCHÜPBACH (1987) homozygous top^1 females produce eggs possessing a ventralized chorion (egg shell) that give rise to a correspondingly ventralized embryo. Eggs from *top* females have a characteristic chorion morphology: rather than possessing two dorsolaterally located dorsal appendages, as do wild type, these eggs bear fused dorsal filaments positioned on the dorsal midline of the egg (Figure 7C). Eggs laid by females of many heteroallelic genotypes exhibit a more extreme degree of ventralization than those produced by top^1 homozygotes. These eggs show progressively greater reductions in the size of their dorsal appendages, with the most extreme chorions lacking all traces of dorsal filament material (Figure 7, E and F). This phenotype resembles that of extreme *gurken* alleles (SCHÜPBACH 1987), although *top* eggs never exhibit the posterior pole abnormalities seen in *gurken*. Like the most extreme *gurken* eggs, severely ventralized *top* eggs are rarely fertilized. Embryos developing within *top* eggs are also ventralized. The ventralmost embryonic tissue, the mesoderm, is expanded and the dorsalmost cells are correspondingly transformed to assume more ventral fates (SCHÜPBACH 1987). This leads to the embryonic cuticle phenotype shown in Figure 7D: the animal produces only ventral cuticle, which is recognizable by its denticles.

In general, the strength of the chorion defects and maternal effect ventralization of the embryo correlate well. In certain female sterile combinations, however, this correspondence is not absolute. For example top^1 homozygotes occasionally lay eggs that possess two dorsal appendages but still produce ventralized embryos. On the other hand, top^1/top^{ED16} females can lay eggs with a single dorsal filament that develop into hatching embryos. Variation between the degree of follicle cell and embryonic defects might arise if one process is directly, and the other only indirectly, regulated by the *top* product. However, our observations

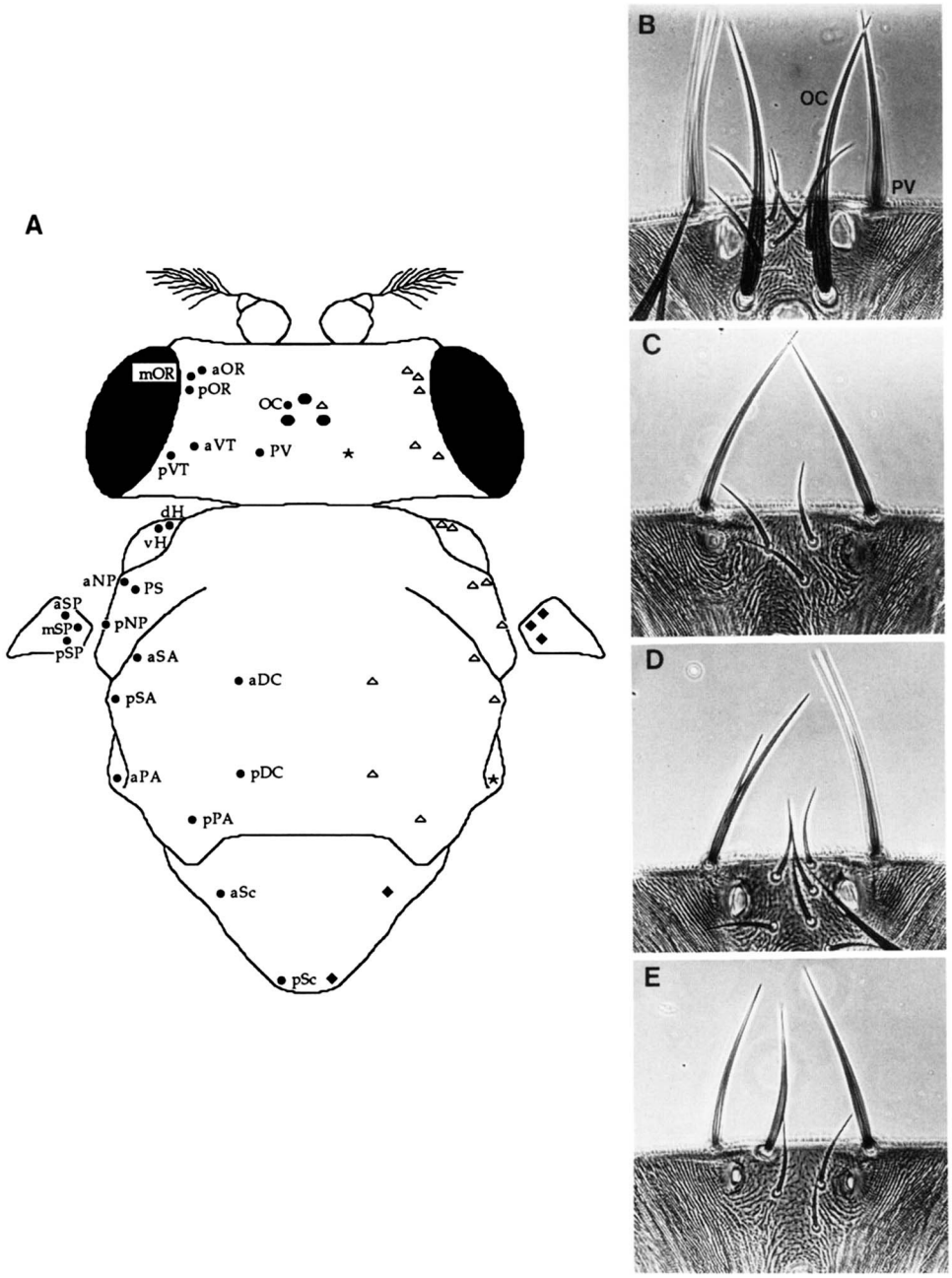

FIGURE 6.—*torpedo* is necessary for the development of the adult bristle pattern. (A) Schematic diagram of the cephalic and thoracic bristle pattern. The name of each bristle is indicated on the left and the manner in which the bristle is altered in *top* mutants is indicated on the right. Diamonds indicate unaffected bristles, triangles indicate eliminated bristles and stars indicate duplicated bristles. Abbreviations are as follows. aOR, anterior orbital bristle; mOR, median orbital bristle; pOR, posterior orbital bristle; OC, ocellar bristle; aVT, anterior vertical bristle; pVT, posterior vertical bristle; PV, postvertical bristle; dH, dorsal humeral bristle; vH, ventral humeral bristle; PS, presutural bristle; aNP, anterior notopleural bristle; pNP, posterior notopleural bristle; aSP, anterior sternopleural bristle; mSP, median sternopleural bristle; pSP, posterior sternopleural bristle; aSA, anterior supraalar bristle; pSA, posterior supraalar bristle; aPA, anterior postalar bristle; pPA, posterior postalar bristle; aDC, anterior dorsocentral bristle; pDC, posterior dorsocentral bristle; aSc, anterior scutellar bristle; pSc,posterior scutellar bristle. (B) Phase contrast photomicrograph of wild-type ocellar (OC) and postvertical (PV) bristles. Note that the anterior-posterior orientation of this panel is opposite that of panel A. (C) The ocellar region of a top^{1}/top^{EB} adult. The two postverticals appear normal, while the ocellar bristles are deleted. (D) Ocellar region of a top^{1}/top^{SH2} animal. The postvertical bristle on the left is partially duplicated. The ocellar bristles are absent. (E) Ocellar region of a top^{1}/top^{2G31} animal. An ectopic postvertical bristle lies between the two normal postverticals. The ocellar bristles are absent.

do not suggest that these two aspects of the oogenesis phenotype are functionally separable, since we have never observed strongly ventralized embryos in wild-type chorions or the converse.

Genetic analysis of *torpedo* function

The phenotypes described above indicate that *top* is required for a variety of temporally and spatially distinct developmental processes. Complementation tests employing embryonic lethal, postembryonic lethal and adult viable *top* lesions as tester alleles have provided insights into the genetic nature of the *top* mutations and the functional organization of the locus.

All *torpedo* alleles analyzed in this study are loss of function mutations: To assess the genetic nature of the available *top* alleles, all mutations were crossed to $Df(2R)top^{3F18}$. The majority of alleles in our collection are recessive zygotic embryonic lethal mutations. The phenotype of mutations exhibiting a severe "flb" embryonic lethal phenotype remains unchanged over the deficiency, suggesting that they represent amorphic (total loss of function) lesions. Alleles producing intermediate or weak "flb" phenotypes usually show a slightly enhanced phenotype in *trans* to $Df(2R)top^{3F18}$; this argues that they are hypomorphic (reduced activity) mutations (Table 1). The semiviable allele top^{CA} and the three lesions that cause postembryonic lethality as homozygotes are all lethal in *trans* to a deletion of the locus. In combination with

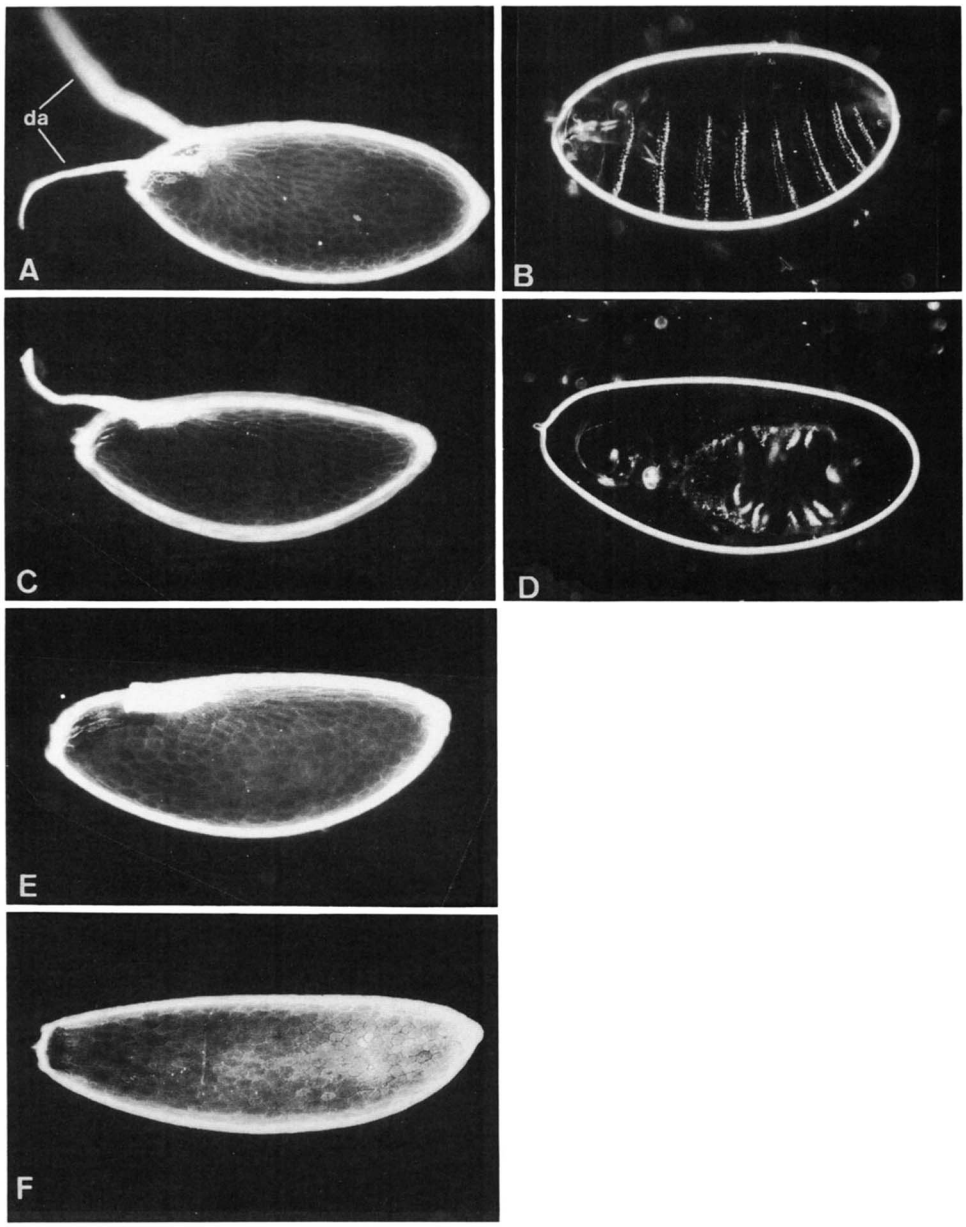

FIGURE 7.—*torpedo* is required for oogenesis. (A) Dark-field photomicrograph of a wild-type chorion (egg shell). The dorsal appendages (da) are positioned on either side of the dorsal midline of the egg and extend anteriorly. (B) Wild-type embryonic cuticle. The highly refractile denticle bands are located on the ventral surface of the embryo. (C) Egg laid by a top^{1}/top^{3C87} female. The egg shell is ventralized: the dorsal filaments are shifted dorsally onto the dorsal midline of the egg where they fuse into a single dorsal appendage. This chorion phenotype is identical to that seen in top^{1} homozygotes. (D) Cuticle of an embryo produced by a top^{1}/top^{1} female. The embryo is ventralized. A patch of cuticle bearing ventral denticles overlies the dorsal surface of the animal. (E) Egg laid by a top^{1}/top^{3C81} female. The mutant egg shell shows a greater degree of ventralization than is seen in eggs produced by flies homozygous for the top^{1} mutation. The dorsal appendages are fused and greatly reduced in size. (F) Egg laid by a top^{1}/top^{CO} heterozygote. This egg is severely ventralized. No dorsal appendage material is visible. The mutant chorion is also larger than a wild-type egg shell, as follicle cells that would have secreted the dorsal appendages assume more ventral fates and contribute to the production of the main body of the chorion.

$Df(2R)top^{3F18}$, two of the homozygous postembryonic lethal mutations exhibit early pupal lethality, while the third, top^{EA}, shows a weak embryonic lethal phenotype. Animals *trans*-heterozygous for top^{CA} the semiviable allele, and the deficiency die late in pupal development as pharate adults (Table 1). The remaining three mutations, top^{1}, top^{CJ} and top^{EE38}, are viable in combination with $Df(2R)top^{3F18}$. All three lesions, however, exhibit much more severe adult pattern defects over the deletion than they do either in the homozygous state or in *trans* to one another (Figures 8 and 9). Thus top^{EA}, top^{CA} and the adult viable alleles also appear to be loss of function mutations. $Df(2R)top^{3F18}$ removes at least 15 polytene chromosome bands. To address the possibility that hemizygosity for genes flanking the *top* locus affects the mutant phenotypes observed in heteroallelic combinations involving this deficiency, all alleles were also examined over the apparent amorphic point mutation top^{CO}. The phenotypes observed in complementation tests with top^{CO} do not differ from those seen over $Df(2R)top^{3F18}$ (data not shown).

***torpedo* possesses distinct embryonic and postembryonic vital activities:** Results obtained in complementation tests with top^{CO} and $Df(2R)top^{3F18}$ suggested that the *top* mutations can be ordered in a simple allelic series in which the severity of a given lesion's lethal phenotype corresponds to the amount of *top* activity it retains. Amorphic mutations exhibit a strong "flb" embryonic lethal phenotype; as the amount of residual top activity is increased, the zygotic lethal phenotypes progress from a moderate embryonic lethal phenotype to a weak "flb" phenotype to pupal lethality. Alleles which result in the least reduc-

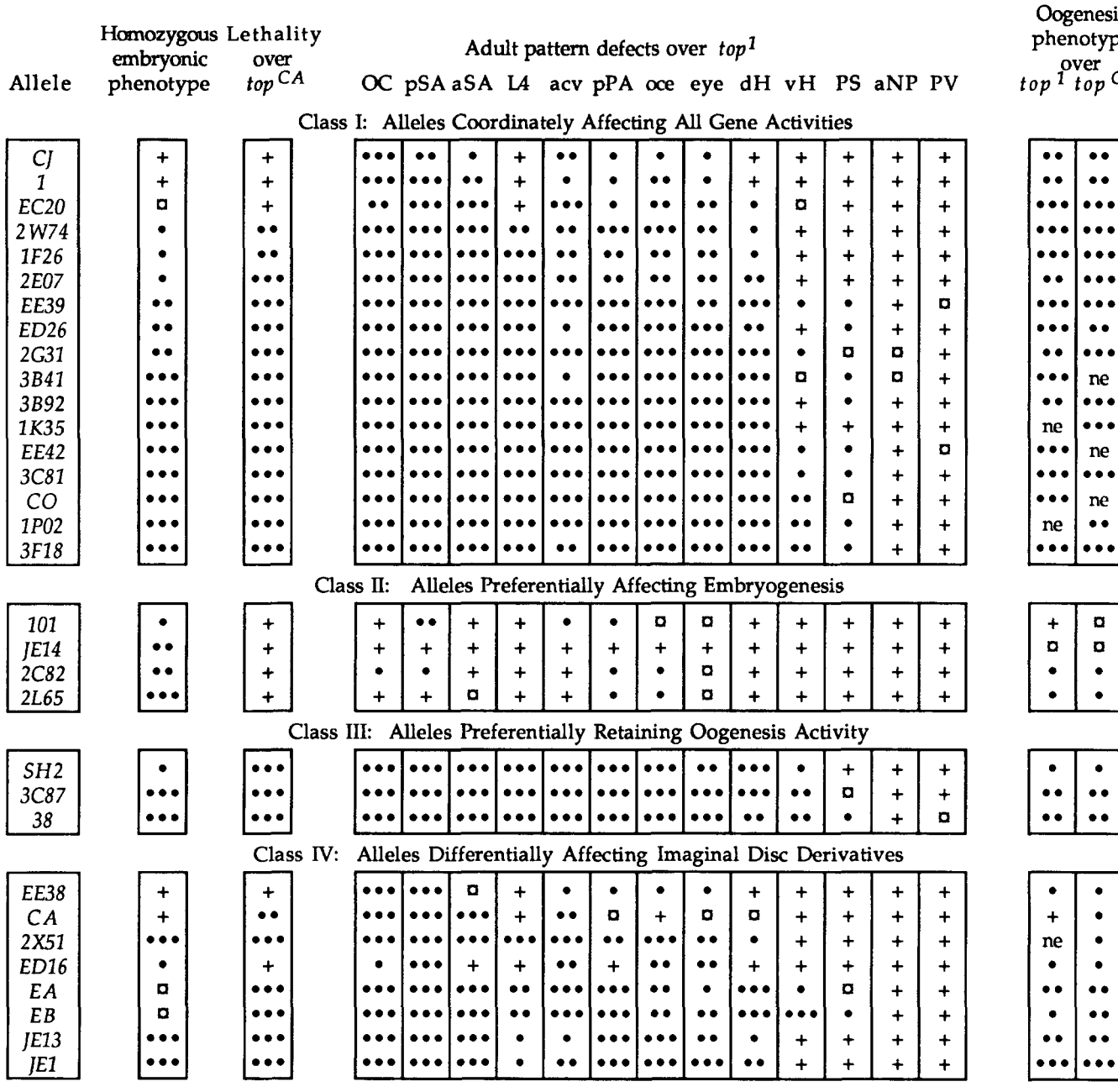

FIGURE 8.—Phenotypic analysis of the *torpedo* alleles. Abbreviations are as follows. L4, fourth longitudinal wing vein; acv, anterior crossvein; oce, ocellus. Refer to Figure 6 for abbreviations of bristle names. Phenotypic quantifications are as follows. Homozygous embryonic phenotype: "+" = adult viable, "⊡" postembryonic lethal, "•" = weak "flb" embryonic lethal, "••" = moderate "flb" embryonic lethal, "•••"= extreme "flb" embryonic lethal. Lethality over *top*CA: "+"= 100–75% survival of *trans*-heterozygous adults, "•" = 74–50% survival, "••" = 49–25% survival, "•••" = 4–0% survival. Oce: "+" = wild-type ocellar morphology, "⊡" = mild, variable size reduction of the ocelli, "•" = mild size reduction, "••" = moderate size reduction, "•••" = extreme size reduction. Eye: "+" = wild-type compound eye morphology, "⊡" = variable, slight eye roughness, "•" = mild eye roughness, "••" = moderate eye roughness, "•••" = extreme eye roughness. Bristles and wing veins: = 0–14% frequency of defects, "⊡" = 15–24% frequency, "•" = 25–49% frequency, "••" = 50–74% frequency, "•••" = 75–100% frequency. Oogenesis: "+" = produces wild-type eggs, "⊡" = occasionally produces eggs with fused dorsal appendages that give rise to mostly normal embryos, "•" = usually produces eggs with fused dorsal appendages that give rise to normal and some weakly ventralized embryos, "••" = produces eggs with fused dorsal appendages that give rise to ventralized embryos, "•••" = produces eggs with fused dorsal appendages that give rise to strongly ventralized embryos and eggs with severely reduced or absent dorsal appendages that are rarely fertilized, "ne" = no eggs laid.

tion of *top* function survive to adulthood over a deficiency and are female sterile.

However, complementation tests performed between top^{CA} and all other *top* alleles indicate that certain mutations do not fit into a simple allelic series. top^{CA} is a semiviable lesion exhibiting pupal lethality in *trans* to a deficiency of the locus. If all alleles in the collection can be arranged in a simple series, muta-

Genotype	OC	pSA	aSA	L4	acv	pPA	eye	dH	vH	PS	aNP	pNP	aPA	aDC	pDC	Egg
CJ / EC20	•••	••	+	+	••	+	+	+	+	+	+	+	+	+	+	••
1 / EC20	••	•••	••	+	•••	•	+	•	+	+	+	+	+	+	+	•••
EE38 /EC20	•••	•••	•••	••	•••	•	+	•••	••	•	+	+	+	+	+	•
CA / EC20	••	•••	•	•	••	••	+	••	+	+	+	+	+	+	+	•
CJ / ED16	••	••	+	+	+	+	•	+	+	+	+	+	+	+	+	•
1 / ED16	•	•••	+	+	•	+	••	+	+	+	+	+	+	+	+	•
EE38 / ED16	••	•••	+	+	•	+	+	+	+	+	+	+	+	+	+	+
CA / ED16	•••	•••	•	□	•	•	□	+	+	+	+	+	+	+	+	ND
CJ / ED26	•••	•••	•••	•••	•••	•	•••	••	+	•	+	+	+	+	+	•••
1 / ED26	•••	•••	•••	•••	•	•••	•••	••	+	•	+	+	+	+	+	•••
EE38 / ED26	•••	•••	•••	•••	•••	•	•	•••	•••	••	+	+	+	+	+	•
CJ / EB	•••	•••	•••	••	•••	••	••	•••	•	•	+	+	+	+	+	ND
1 / EB	•••	•••	•••	••	•••	•••	••	•••	•••	•	+	+	+	+	+	+
EE38 / EB	•••	•••	•••	•••	•••	•••	•	•••	•••	•••	•	•	+	+	+	ND
CJ / 38	•••	•••	•••	•••	•••	•	•••	••	+	••	+	+	+	+	+	••
1 / 38	•••	•••	•••	•••	•••	•••	•••	•••	••	•	+	+	+	+	+	••
EE38 / 38	•••	•••	•••	•••	•••	•	•	•••	•••	••	+	+	+	+	+	ND
CJ / 3F18	•••	•••	•••	•••	•••	•••	••	•••	•••	•	+	+	+	+	+	•••
1 / 3F18	•••	•••	•••	•••	••	•••	•••	•••	••	•	+	+	+	+	+	•••
EE38 / 3F18	•••	•••	•••	•••	•••	••	•	•••	•••	•	•	•	□	+	+	•

FIGURE 9.—The adult viable *torpedo* mutations show qualitative phenotypic differences. Adult phenotypes of top^{CJ}, top^{1}, top^{EE38} and top^{CA} in combination with representative tester alleles. top^{CA}/top^{ED26}, top^{CA}/top^{EB}, top^{CA}/top^{38} and top^{CA}/top^{3F18} animals do not survive to adulthood. Abbreviations are the same as in Figure 8, with the addition of the following: "*IND*" = phenotype not determined.

tions showing a severe embryonic lethal phenotype (presumptive amorphic alleles) should die over top^{CA}, lesions showing moderate "flb," weak embryonic lethal and pupal lethal phenotypes might show intermediate viability that increases with decreasing allelic strength and the adult viable alleles should survive well in combination with top^{CA}. This prediction was not entirely borne out. Although most alleles behave as expected, four embryonic lethal mutations with different terminal phenotypes ranging from weak to strong (top^{101}, top^{2C82}, top^{JE14}, top^{2L65}) are fully viable over top^{CA} (Figures 8 and 10). Thus these four mutations do not appear to fit into a simple allelic series based on quantitative differences in *top* activity; rather, it appears that these alleles retain a *top* activity needed for pupal survival while they reduce a gene activity required for embryonic development.

To demonstrate that this complementation does not reflect a specific interaction with top^{CA}, the four exceptional alleles were also crossed to the pupal lethal lesions top^{EB} and top^{EC20}. As controls, top^{EB} and top^{EC20} were also crossed to three embryonic lethal alleles showing the expected severely reduced viability over top^{CA}. Both top^{EB} and top^{EC20} die in *trans* to the control mutations; however, both pupal lethal mutations survive to adulthood over top^{101}, top^{2C82}, top^{JE14} and top^{2L65} (Table 2). Since these four embryonic lethal lesions complement the pupal lethality of several *top* mutations, it is unlikely that complementation is due to allele-specific interactions. Rather, the exceptional lesions, while clearly deficient for *top*'s embryogenesis activity, still retain gene activity needed for pupal viability. As top^{101}, top^{2C82}, top^{JE14} and top^{2L65} all show some reduction in viability in combination with top^{EB}, they are not completely wild-type for pupal activity (Table 2). Thus these mutations preferentially, but not exclusively, affect a gene function required for embryogenesis.

The adult viable *torpedo* alleles show qualitative differences in their effects on imaginal disc patterning and oogenesis: To assess the nature and severity of the imaginal disc and oogenesis phenotypes of the *top* lesions, and to elucidate how the adult defects of each mutation correlate with one another and with the mutation's embryonic lethal and pupal lethal phenotypes, the *top* alleles were examined in *trans* to top^{1}, top^{CJ} and top^{EE38}, mutations which are viable over $Df(2R)top^{3F18}$. In addition, we have determined the adult phenotypes of the viable top^{CA} heteroallelic combinations (Figures 8, 9 and 10).

Over a given *top* allele, top^{CJ} generally produces the weakest imaginal disc pattern defects, top^{1} shows a more severe phenotype and top^{EE38} and top^{CA} exhibit the most extreme overall phenotypes (Figures 9 and

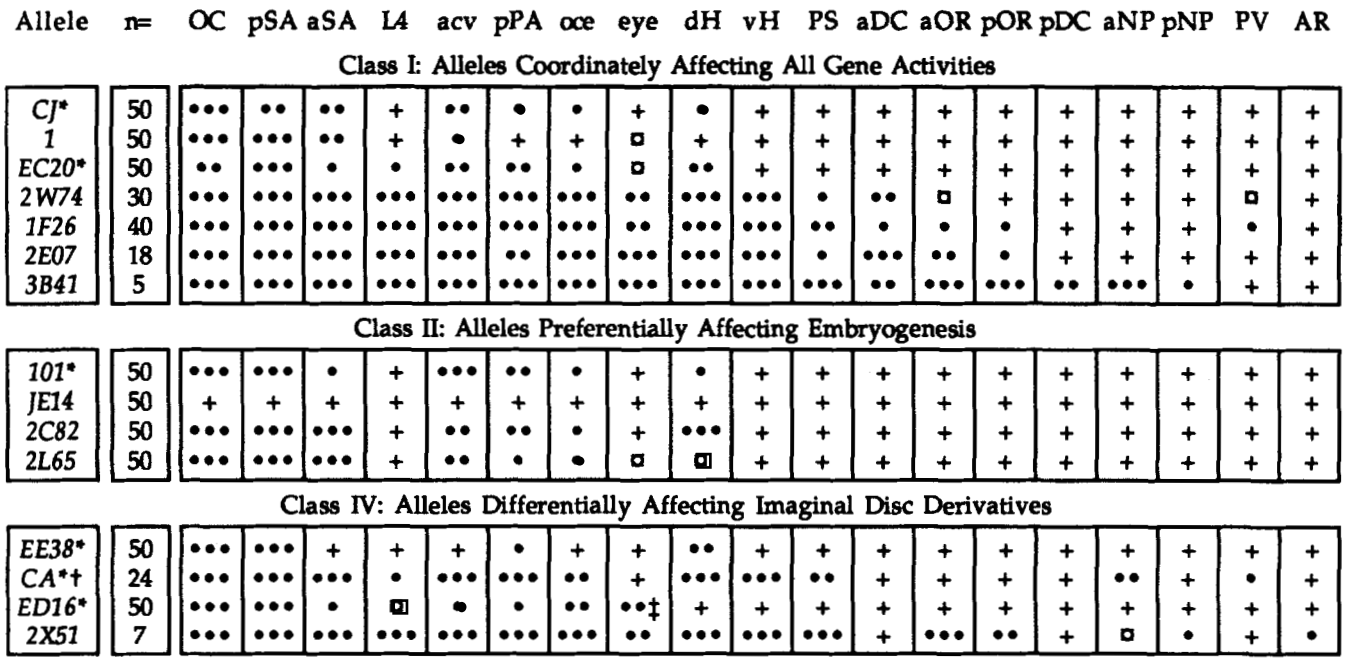

FIGURE 10.—Phenotypes of the *torpedo* alleles in combination with top^{CA}. Abbreviations are the same as in Figures 8 and 9, with the addition of the following: "n" = the number of adults examined for each genotype; AR, arista. "*" = the allele was tested over a *b pr cn* top^{CA} recombinant chromosome rather than the original *b pr cn sca* top^{CA} chromosome; "†" = the cross was performed at room temperature rather than 25°; top^{CA}/top^{ED16} females exhibit more extreme eye roughening than top^{CA}/top^{ED16} males.

TABLE 2

Certain embryonic lethal *torpedo* mutations complement pupal lethal alleles

Cross			*mutant* / *balancer*	*mutant* / *mutant*	Survival rate of *trans*-heterozygote (%)
top^{CA}	×	top^{2W74}	216	35	32
top^{CA}	×	top^{2E07}	219	19	17
top^{CA}	×	top^{3B92}	201	1	1
top^{CA}	×	top^{101}	278	154	**111**
top^{CA}	×	top^{2C82}	271	171	**126**
top^{CA}	×	top^{JE14}	205	110	**107**
top^{CA}	×	top^{2L65}	241	129	**107**
top^{EC20}	×	top^{2W74}	124	0	<2
top^{EC20}	×	top^{2E07}	123	0	<2
top^{EC20}	×	top^{3B92}	135	0	<2
top^{EC20}	×	top^{101}	137	43	**63**
top^{EC20}	×	top^{2C82}	139	80	**115**
top^{EC20}	×	top^{JE14}	125	97	**155**
top^{EC20}	×	top^{2L65}	154	77	**100**
top^{EB}	×	top^{2W74}	49	0	<5
top^{EB}	×	top^{2E07}	139	0	<2
top^{EB}	×	top^{3B92}	172	0	<2
top^{EB}	×	top^{101}	111	6	**11**
top^{EB}	×	top^{2C82}	116	13	**22**
top^{EB}	×	top^{JE14}	120	52	**87**
top^{EB}	×	top^{2L65}	120	15	**25**

top^{2W74}, top^{2E07} and top^{3B92} are class I alleles with weak, weak and severe embryonic lethal phenotypes, respectively.

top^{101}, top^{2C82}, top^{JE14} and top^{2L65} are class II alleles with weak, moderate, moderate and strong embryonic lethal phenotypes, respectively.

10) (data not shown). Since top^{CA} is lethal over a deficiency of the locus and in combination with many embryonic lethal *top* lesions, it represents the most severely affected adult viable allele. Systematic exceptions to this ordering do exist. top^{CJ} combinations exhibit higher frequencies of ocellar bristle defects than do the corresponding top^{1} heterozygotes and often show more extreme oogenesis phenotypes (Figures 8 and 9), while top^{EE38} and top^{CA} heteroallelic animals usually exhibit substantially weaker eye and oogenesis abnormalities and more extreme wing and bristle defects than do the corresponding top^{1} or top^{CJ} *trans*-heterozygotes (Figures 9 and 10). As these differences between the adult viable lesions are observed over a variety of tester mutations, they are more likely due to qualitative differences between top^{1}, top^{CJ}, top^{EE38} and top^{CA} than to allele-specific interactions between the adult viable and tester alleles or to differences in genetic background. Thus the overall phenotype of a given adult viable allele becomes more extreme as the residual activity of the tester allele decreases, and the regional specificities of its defects are reflected in each heteroallelic combination.

Complementation tests with the adult viable alleles allow us to measure how each *top* mutation affects the development of the imaginal disc derivatives and oogenesis. These phenotypes also provide a means of confirming the classification of *top* alleles based on zygotic lethal phenotypes and the ability to complement the pupal lethality of top^{CA}. The most easily scored adult pattern defects (eye roughening, dis-

rupted wing venation, bristle abnormalities and female sterility) were used for the phenotypic analysis.

***torpedo* mutations can be divided into phenotypic classes:** By comparing each allele's zygotic lethal phenotype (an indicator of its embryogenesis activity) to its rate of survival in *trans* to top^{CA} (a measure of its pupal vital activity), the severity of its adult defects over top^{1} (a measure of its imaginal disc activities) and its ability to complement the female sterility of top^{1} and top^{CJ} (an indicator of its oogenesis activity), the *top* mutations can be divided into four classes (Figure 8). Most lesions fall into one of three allelic series (I–III). Within each series alleles may be ranked according to the amount of *top* activity they retain; however, the series are distinguished by apparent qualitative differences in the degree to which their member alleles affect the various *top* activities. Members of the fourth class of mutations, in contrast, cannot be ordered in an allelic series.

The 17 class I mutations (top^{CJ}-$Df(2R)top^{3F18}$) appear to reduce all gene activities in a uniform manner. When these mutations are ordered by the severity of their homozygous zygotic lethal phenotypes, it can be seen that an allele's ability to complement top^{CA} (which should be proportional to the amount of pupal vital activity an allele possesses), the strength of adult morphological defects produced in *trans* to top^{1} and the ability to complement the female sterility of top^{1} and top^{CJ} correspond fairly well to the severity of its homozygous lethal phenotype. This ordering further indicates that the relative sensitivities of eight macrochaetae, the fourth longitudinal vein, the anterior crossvein, the compound eye and the ocellus fall into a fairly consistent hierarchy (Figure 8). Since $Df(2R)top^{3F18}$ by all criteria falls into this class of *top* lesions, class I presumably represents mutations that reduce all gene activities more or less uniformly.

The class II lesions, top^{101}, top^{JE14}, top^{2C82} and top^{2L65}, preferentially exhibit defects in embryogenesis. Although these alleles are embryonic lethals, they survive in *trans* to top^{CA}, top^{EB} and top^{EC20}; they therefore retain considerably more pupal vital activity than would be predicted from their homozygous embryonic lethal phenotypes (Table 2, Figure 8). These four mutations also exhibit very mild adult pattern and oogenesis abnormalities over top^{1}, and can be ordered in their own allelic series.

Class III mutations, top^{SH2}, top^{38} and top^{3C87}, differ from alleles of the first class by preferentially retaining oogenesis activity. These three lesions show significantly weaker female sterile phenotypes—both in combination with top^{1} and top^{CJ}—than do class I alleles showing equivalent homozygous lethal phenotypes, survival over top^{CA} and adult pattern defects. The three lesions can be ordered in a hypomorphic series, with top^{SH2} and top^{3C87} representing the weakest and strongest alleles, respectively.

Class IV mutations (top^{EE38}-top^{JE1}) exhibit phenotypes suggesting that they preferentially lack or retain gene activity required for particular developmental processes. top^{EE38} and top^{CA} (see Figures 9 and 10) exhibit milder eye defects than would be expected from the severity of their wing and bristle abnormalities, whereas top^{ED16} shows the converse phenotype, enhanced eye defects. top^{EE38} and top^{CA} also appear to retain significant oogenesis activity. top^{EA} and top^{EB} preferentially produce bristle defects and show enhanced lethality in *trans* to top^{CA}. On the other hand, top^{2X51} seems to preferentially retain eye and bristle activity. When compared to class I mutations producing eye and bristle defects of equivalent severity, top^{JE13} and top^{JE1} exhibit an enhanced zygotic embryonic lethal phenotype and a decreased disruption of wing venation (Figure 8).

Thus a phenotypic examination of the *top* alleles reveals that several developmental processes dependent upon *top* are differentially, though not exclusively, affected by lesions in the gene. These include the functions required for embryonic development, the differentiation of the eye, wing and bristles and oogenesis This phenomenon may reflect the differential mutability of regulatory or structural elements of the *top* locus, cell type-specific stability of mutant gene products, cell type-specific reduction of mutant protein activity or some combination of the above.

DISCUSSION

It has recently been shown that the *torpedo* (*top*) gene of Drosophila encodes the fruitfly homolog of the vertebrate EGF receptor and *neu* proto-oncogene, DER (PRICE, CLIFFORD and SCHÜPBACH 1989; SCHEJTER and SHILO 1989). Previous molecular studies have shown that two alternately spliced mRNAs are transcribed from this locus throughout the life of the fly (LEV, SHILO and KIMCHIE 1985; WADSWORTH, VINCENT and BILODAEU-WENTWORTH 1985; SCHEJTER *et al.* 1986; KAMMERMEYER and WADSWORTH 1987). Both transcripts are expressed in all tissues of the embryo, in the developing eye-antennal, wing and genital imaginal discs of the larvae, in the adult nervous system and in the follicle cell epithelium of the ovary (SCHEJTER *et al.* 1986; KAMMERMEYER and WADSWORTH 1987). We have genetically analyzed 32 *top* mutations. This analysis has allowed us to assay the spatial and temporal requirements for *top* activity and has revealed the functional complexity of the gene.

Based on a comparison of their phenotypes, the *top* alleles can be divided into several broad categories: those affecting all gene activities uniformly, those preferentially affecting embryogenesis, those affect-

ing oogenesis to a lesser degree than other developmental processes and those differentially affecting the development of specific imaginal disc derivatives. This analysis suggests that *top* possesses differentially, though not independently, mutable functions regulating processes required for oogenesis, embryogenesis, pupal development, wing venation, the formation of the compound eye and ocellus and the development of sensory bristles.

The phenotypes of the *top* alleles argue against the idea that the gene possesses independent functional domains. If, for example, there existed an independently mutable domain required only for oogenesis, we should have found *top* lesions causing only female sterility. No allele of this kind has been isolated. The absence of mutations exclusively affecting oogenesis is not due to the mutagenesis protocols, since the 14 alleles isolated in our screens were selected as female sterile lesions. All proved to affect imaginal disc development, and many are zygotic lethals. By the same token, 18 alleles were selected as zygotic lethals, yet all affect oogenesis (Figure 8). A similar argument can be made against the existence of an independent domain encoding the embryonic vital activity. None of the *top* alleles selected as zygotic lethal mutations exclusively affects embryogenesis (Figure 8). Thus, although the oogenesis and embryogenesis activities of *top* can be preferentially affected by lesions at the *top* locus, they were not mutated independently of the remaining gene activities in any allele in our sample.

Given the expression pattern of *top* and the homology of its product to the vertebrate EGF receptor, the phenotypic complexity exhibited by *top* alleles can be explained in various ways. One possible basis for the gene's differentially mutable activities is that it encodes multiple products. Sequence analysis of cDNA clones corresponding to the two *top* transcripts has shown that the predicted products of the two mRNAs differ at their amino termini (LEV, SHILO and KIMCHIE 1985; SCHEJTER and SHILO 1989). If each protein functions primarily in a subset of the developmental process requiring *top* but also acts in a secondary capacity in all remaining *top*-dependent processes, point mutations in the locus can preferentially, but not exclusively, affect gene functions. This model further predicts that there will exist one more class of *top* point mutations than there are gene products: a class of alleles specific to each alternate form of the protein and an additional class of mutations altering the region of the gene common to both products. Our collection of alleles does contain lesions affecting all *top* activities (the class I alleles) and lesions that preferentially affect embryogenesis (the class II alleles). Other alleles appear to preferentially disrupt the development of the compound and simple eyes (top^{ED16}) or the bristles (top^{EA} and top^{EB}). The existence of more than three classes of *top* lesions argues that this explanation for differential mutability is not sufficient to account for *top*'s phenotypic diversity.

Another possible source of phenotypic complexity is that one or both products of the *top* locus may interact with several tissue-specific ligands or substrates. By functional analogy to the mammalian EGF receptor, the extracellular domain of a *top* protein might interact with a variety of tissue-specific signal molecules, and its intracellular tyrosine kinase domain may phosphorylate a variety of cellular proteins. Lesions in *top* could then alter the conformation of one or both domains so that binding to one ligand or substrate is severely disrupted while the remaining interactions are affected to lesser extents.

A third possibility is that some *top* mutations interfere with the stability or activity of the gene products in a tissue- or cell-specific manner: certain mutant mRNAs or proteins may be differentially destabilized in a subset of the tissues in which they are expressed and the activity of mutant proteins may vary in a cell-specific fashion. A final source of phenotypic complexity may be genetic background. Background mutations in some *top* stocks may modify the expression or activity of the mutant gene at specific stages of development or in specific tissues of the fly. These mechanisms for the gene's functional complexity are not mutually exclusive, and the genetic data does not favor any particular explanation. The molecular analysis of specific *top* mutations may provide insight into this question.

The high degree of structural similarity existing between the predicted *top* protein products and the vertebrate epidermal growth factor receptor argues that *top* is involved in the reception of extrinsic signals during Drosophila development. The nature of the defects seen in *top* mutants provide insight into how tissues in the developing fly respond to these signals.

Analysis of the oogenesis defect of *top* suggests that this gene mediates intercellular communication in the ovary leading to the establishment or maintenance of cellular identities. During oogenesis *top* activity is required to promote dorsal follicle cell fate; maternally expressed *top* product also acts through the *dorsal-Toll* group of genes to regulate the dorsoventral pattern of the embryo (SCHÜPBACH 1987; PRICE, CLIFFORD and SCHÜPBACH 1989).

The zygotic function of *top* in the embryo is very different from its role in oogenesis: early zygotic gene activity is not required for the initial establishment of the embryonic body pattern. The head and thoracic segments of embryos homozygous for severe zygotic lethal *top* alleles appear to form normally, but they degenerate during later development (SCHEJTER and SHILO 1989; R. J. CLIFFORD and T. SCHÜPBACH, unpublished results). This defect is associated with ex-

tensive cell death (PRICE, CLIFFORD and SCHÜPBACH 1989; R. J. CLIFFORD and T. SCHÜPBACH, unpublished). Thus the zygotic embryonic activity of *top* is required for the viability of particular embryonic tissues.

The postembryonic defects of *top* argue that the gene is required for the growth of the imaginal discs during larval development and the subsequent patterning of cuticular structures derived from the discs during pupation. The absence or duplication of particular bristles on the head and thorax, the production of supernumerary sex comb teeth, the elimination of the tarsal claw, the deletion of specific wing veins and a disruption of the compound eye pattern are seen in animals of viable *top* genotypes. These position-specific defects suggest that reductions or alterations in *top* activity may cause cells to assume incorrect identities by interfering with intercellular communication, which is known to play an important role in the patterning of bristles on the cuticle (GARCIÀ-BELLIDO 1981; MOSCOSO DEL PRADO and GARCIÀ-BELLIDO 1984), the patterning of the compound eye (TOMLINSON and READY 1987a, b; HAFEN *et al.* 1987; REINKE and ZIPURSKY 1988) and in wing venation (GARCIÀ-BELLIDO 1977; RIPOLL *et al.* 1988). Defects observed in animals of more severe *top* genotypes are consistent with this gene mediating the reception of signals required for cellular proliferation or viability in the imaginal discs. Larvae of the early pupal lethal genotype $top^{EC20}/Df(2R)top^{3F18}$ suffer severe reductions in the size of the wing and haltere imaginal discs and a moderate size reduction in the portion of the eye-antennal disc that gives rise to the eye, ocellus and head capsule.

There apppears to be a correspondence between the growth and differentiation abnormalities of the imaginal discs. In the mutant genotype examined, wing and haltere discs are nearly absent, while the eye portion of the eye-antennal disc is somewhat reduced in size and the leg imaginal disc and the region of the eye-antennal disc giving rise to the antenna appear morphologically normal. In the adult, pattern of the wing is more sensitive to disruption by reductions in or alterations of the *top* product than is the patterning of the eye; the development of the compound eye, in turn, is more sensitive to disruption than the development of the antenna or leg. In addition, a *top* allele's ability to complement the pupal lethality of top^{CA}, which presumably corresponds to the severity of its imaginal disc growth defects, always correlates with the overall strength of its adult pattern defects. This correlation holds true for lesions of all phenotypic classes. This correspondence lends further support for the idea that *top* participates in a process through which the growth and pattern formation of the imaginal discs are intimately linked.

The good correlation seen between the imaginal disc growth and patterning abnormalities of *top* mutants can be explained in several ways. First, spatially restricted adult pattern defects may arise from localized defects in cell proliferation or viability. Since the adult defects presumably result from a less severe reduction or alteration of the *top* product than do the imaginal disc growth defects, the adult pattern abnormalities would occur in regions of the disc showing the greatest requirement for the putative proliferation or maintenance signal transduced by *top*. Another possibility is that the growth defects of the imaginal discs are the consequence of alterations in cell identities. The loss of certain cell fates in the disc could lead to a reduction in cell proliferation and a concomitant reduction in the final size of the imaginal disc. A third possiblity is that growth and patterning of the discs are unrelated processes that both require the *top* gene product.

In summary, the range of defects seen in *top* mutants suggests that the gene participates in a variety of developmental processes. In the ovary, *top* is required for the establishment or maintenance of cellular identities, in the embryo, it is needed for the viability of certain tissues and, in the imaginal disc, the gene is necessary for growth and patterning. Further genetic and biochemical analysis of *top* should shed light on the functional relationships of these diverse physiological processes and the nature of the signals that trigger them.

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