GENETIC AND PHENOTYPIC ANALYSIS OF DOMINANT *ENHANCERS OF TORPEDO* ON THE SECOND AND THIRD CHROMOSOMES OF *DROSOPHILA MELANOGASTER*.

by

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B. Sc., Simon Fraser University, 1991

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in the Department

of

Biological Sciences

SIMON FRASER UNIVERSITY

February 1995

APPROVAL

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Degree: **Master of Science**

Title of Thesis:

GENETIC AND PHENOTYPIC ANALYSIS OF DOMINANT *ENHANCERS OF TORPEDO* ON THE SECOND AND THIRD CHROMOSOMES OF *DROSOPHILA MELANOGASTER*

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Title of Thesis/Project/Extended Essay

Genetic and Phenotypic Analysis of Dominant Enhancers of torpedo on the Second and Third Chromosomes of *Drosophila melanogaster*.

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March 31, 1995
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Abstract.

The *torpedo* tyrosine kinase gene of *Drosophila melanogaster* encodes the homologue of the vertebrate Epidermal Growth Factor receptor (Price *et al.*, 1989; Schejter and Shilo, 1989). The activity of the *torpedo* gene product is required for embryogenesis, oogenesis, pupal viability, the development of certain imaginal discs and for the patterning of specific imaginal disc derivatives (Schüpbach, 1987; Clifford and Schüpbach, 1989). To identify genes which interact with the *torpedo* locus, genetic screens designed to generate mutations which dominantly enhance phenotypes associated with mutations in *torpedo* were conducted. A total of 25 such mutations, called *Enhancers of torpedo*, or *E(top)*s, were recovered. Twenty mutations were mapped to the second chromosome; five were mapped to the third chromosome.

In Section I, both the genetic and phenotypic characterization of the 20 *E(top)*s which were mapped to the second chromosome is presented. The precise number of genes represented by these 20 mutations is currently unknown. However, preliminary results indicate that at least one locus distinct from *torpedo* has been identified.

In Section II, the characterization of the five *E(top)*s located on the third chromosome is presented. Complementation tests and phenotypic data are consistent with the idea that each of these mutations identifies a gene. Three of these mutations, *E(top) 2-21*, *E(top) D105* and *E(top) K24* (which has been renamed *Bare naked fly*), also behave as dominant enhancers of a gene called *Hairless*. Genetic map positions have been determined for these three mutations. The *E(top) 2-21* mutation was localized to a position of 15.9 map units, the *E(top) D105* locus was assigned to a position of 87.1 map units, and the *Bare naked fly* mutation was mapped to a position of 64.2 map units.

for Karen

There are FOUR lights.

Acknowledgements.

There are a number of people who have contributed in a variety of ways to the successful completion of this thesis and to the enjoyment of the time I have spent in the Price Laboratory. I would like to thank my Senior Supervisor, Dr. James V. Price, for his helpful comments on drafts of this thesis and for granting me the distinction of being his charter graduate student. Thanks also to Drs. David L. Baillie, Charlie M. Boone and Bruce P. Brandhorst for serving as members of my Supervisory and Examination Committees.

I am grateful to my parents for their support and understanding throughout this endeavour. I would also like to thank the following people for their friendship: Lois Schwartz, Marlow Pellatt, Ashton Breitkreutz (whose assistance with the multitude of complementation crosses is greatly appreciated), Gilles Detillieux, Megan Levings, Salim Hanna, Betty Tang, Brad Barbazuk, Jacquie Schein, Norm Franz and Ruth Davenport.

I would like to offer posthumous thanks to Ludwig van Beethoven, Richard Strauß and Richard Wagner, whose sublime music served as both a focus and a source of inspiration during the writing of this thesis.

I am particularly grateful to the following individuals for their grand friendship and support over the years: Kathleen Fitzpatrick [whom I thank for her assistance in the mapping of the *E(top) D76* mutation], Markus Heinrichs, Zenovia Ursuliak, Marco Marra and Sharon "M". Gorski. No one could hope for better friends.

A very special "Thank you" is extended to Karen Ann Rempel, who not only found the *Bare naked fly* mutation, but who has also been a most outstanding and true friend, having provided a great deal of emotional support and encouragement along the way. I cannot thank her enough.

Table of Contents.

Table of Contents. (continued)

Table of Contents. (continued)

List of Tables.

List of Figures.

Introduction.

Receptor tyrosine kinase (RTK) signal transduction pathways are important in the development of all eukaryotes. RTKs are required in a wide variety of processes, participating in oogenesis, wound healing, embryogenesis, the insulin response and morphogenesis. In those eukaryotes which have been studied, these signal transduction pathways have been found to be highly conserved, with their molecular components having been identified in a variety of organisms. These same components have also been found to act in multiple pathways within a given organism. See Figure 1 for a generalized RTK signal transduction pathway.

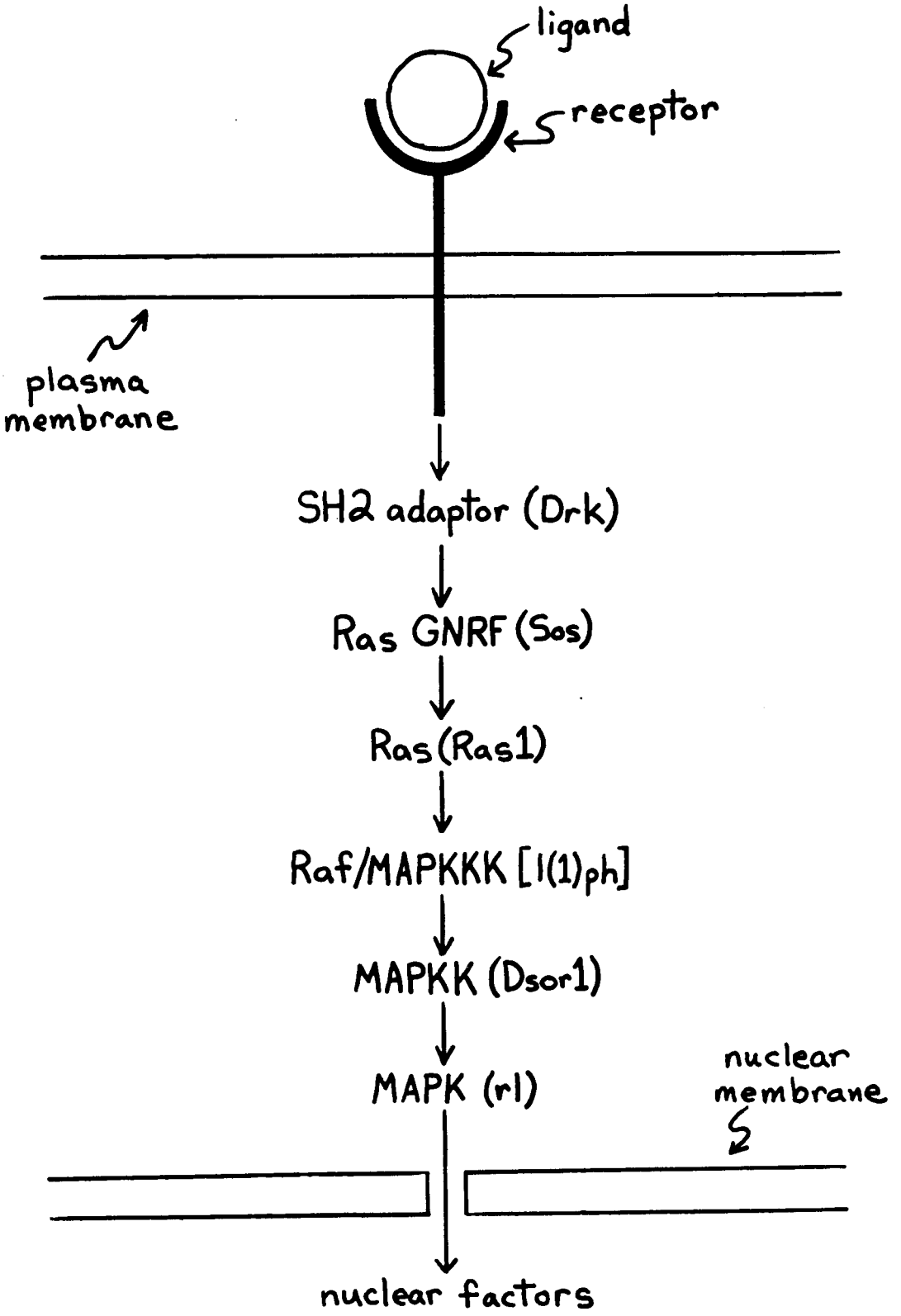
Many questions still remain to be answered in the characterization of various RTKs and their respective pathways. It is of considerable importance to identify the ligands for these receptors as well as their downstream components. As these components are capable of acting in several distinct developmental processes, it is desirable to examine possible relationships between two or more different pathways. The common fruit fly, *Drosophila melanogaster*, represents an organism particularly well suited for the genetic and molecular analysis of signal transduction pathways.

The *torpedo* tyrosine kinase gene of *Drosophila melanogaster* encodes the homologue of the vertebrate epidermal growth factor (EGF) receptor (Price *et al.*, 1989; Schejter and Shilo, 1989). The activity of the *torpedo* gene product is required for oogenesis, embryogenesis, the development of certain imaginal discs and for the patterning of specific imaginal disc derivatives (Schüpbach, 1987; Clifford and Schüpbach, 1989). Mutations in *torpedo* have been recovered which behave as embryonic lethals, produce maternal effect phenotypes in the eggs and embryos derived from mutant females and produce defects in adult structures, particularly the compound eye and the wing (Schüpbach, 1987; Baker and Rubin, 1989; Clifford and Schüpbach, 1989; Price *et al.*, 1989; Schejter and Shilo, 1989; for review see Shilo and Raz, 1991).

Figure 1.

Generalized Signal Transduction Pathway for Receptor Tyrosine Kinases.

Upon binding its ligand, a tyrosine kinase receptor becomes activated and mediates a molecular signaling cascade, the ultimate goal of which is to affect gene expression in the nucleus. The downstream components participating in the transmission of this signal , listed in sequence, are as follows (symbols appearing in parentheses are the names of the homologous proteins in *Drosophila melanogaster*). An SH2 adaptor protein (Downstream of receptor kinases, or Drk) couples the activated receptor to a GNRF/guanine nucleotide releasing factor (Son of sevenless, or Sos), which serves to facilitate the binding of GTP to a Ras molecule (Ras1), resulting in the activation of Ras. Ras, in turn, activates a mitogen-activated protein kinase kinase kinase/MAPKKK [l(1)polehole/l(1)ph], also known as Raf (Ras-activated factor). Downstream of MAPKKK is MAPKK (*Drosophila* suppressor of raf, or Dsor1) which activates a MAPK molecule (rolled). From this point, the signal is transmitted to the nucleus where the activity of target genes is modulated. The torso, sevenless and torpedo/EGF receptors of *Drosophila* all use this molecular cascade in their respective signal transduction pathways. Other molecules may function downstream of MAPK but have yet to be identified. See text for more detailed discussion and description of specific pathways.


ligand
receptor
plasma membrane
SH2 adaptor (Drk)
Ras GNRF (Sos)
Ras (Ras1)
Raf/MAPKKK [l(1)ph]
MAPKK (Dsor1)
MAPK (rl)
nuclear membrane
nuclear factors

***torpedo* is Required for Embryonic Development.**

Wild type embryos display eight distinct rows of ventral denticle bands. In a screen for embryonic lethal mutations on the second chromosome, one mutation was recovered which resembled a curled up mass of dorsal cuticle lacking both the ventral hypoderm and the ventral denticle belts. Given the phenotypic nature of the mutation, the name *faint little ball* (*flb*) was given to the locus (Nüsslein-Volhard *et al.*, 1984). The *flb* embryo is arrested in germ band extension, hence its curled-up appearance. In addition to its cuticle defects, the *flb* embryo also lacks well-defined head structures and shows major disruption of the embryonic central nervous system (CNS) (Clifford and Schüpbach, 1989, 1992; Price *et al.*, 1989; Schejter and Shilo, 1989; Zak *et al.*, 1990; Raz and Shilo, 1992, 1993). It has since been shown that *torpedo* is allelic to the *faint little ball* locus (Price *et al.*, 1989).

The phenotypes of the *flb* embryo are similar to those seen with mutations in a group of genes collectively referred to as the *spitz* group. The members of this group include *spitz*, *Star*, *rhomboid*, *sichel*, *single-minded*, *pointed* and *ocelliless*. When mutant, all of these genes produce defects in the ventral cuticle as well as in the CNS, and by virtue of the cuticle and CNS defects displayed by the *flb* embryo, *torpedo* can also be included as a member of the *spitz* group (Jürgens *et al.*, 1984; Nüsslein-Volhard *et al.*, 1984; Wieschaus *et al.*, 1984; Mayer and Nüsslein-Volhard, 1988; Wieschaus *et al.*, 1992; Kim and Crews, 1993).

The ventral cuticle and CNS defects of *flb* closely resemble those of *spitz*, *Star* and *rhomboid*, implying that these genes may be interacting in some manner. (Raz and Shilo, 1992, 1993). Embryos double-mutant for weak *flb* alleles and *spitz* or *Star* show a synergistic phenotype in that the presence of either *spitz* or *Star* in a weak *flb* background were able to produce a severe *flb* phenotype, a result which would suggest that *spitz*, *Star* and *torpedo* are participating in some common signal transduction pathway involved in

embryonic development (Raz and Shilo, 1993). The *spitz* gene has been cloned and has been found to encode an EGF-like molecule similar to TGFα, making the *spitz* gene product an excellent candidate ligand for the torpedo/EGF receptor (Rutledge *et al.*, 1992). *Star* has also been cloned, and it encodes a novel protein with a putative transmembrane domain (Higson *et al.*, 1993; Kolodkin *et al.*, 1994). In addition, the *rhomboid* locus has been cloned and it was also shown to encode a transmembrane protein (Bier *et al.*, 1990). With the phenotypic similarities of the *torpedo/flb*, *spitz*, *Star* and *rhomboid* mutations, it has been proposed that these four genes are interacting with each other to regulate one or more developmental processes. Ruohola-Baker *et al.* (1993) and Sturtevant *et al.* (1993) have suggested that the *torpedo*, *spitz*, *Star* and *rhomboid* gene products act together, such that rhomboid, and possibly Star, may serve to facilitate spitz-activated/torpedo-mediated signaling.

***torpedo* is Required for Dorsoventral Signaling Processes in Oogenesis.**

The mature egg chamber of *Drosophila* consists of the germ line-derived oocyte and its accompanying complex of 15 nurse cells, all surrounded by the somatically-derived follicle cell epithelium. The follicle cells secrete the vitelline membrane and the eggshell, which together comprise the chorion. There is communication between the oocyte and the follicle cell epithelium which dictates the dorsoventral polarity within the egg chamber. A group of mutations has been described which affect the proper establishment of the dorsoventral polarity of both the eggshell and the embryo (for review, see Schüpbach *et al.*, 1991).

The original *torpedo* mutation, top^1, was recovered in a screen for recessive female sterile mutations. The top^1 mutation causes the ventralization of both the eggshell and the embryo (Schüpbach, 1987). In a wild type eggshell, two chorionic processes called dorsal appendages are present at the dorsoanterior region of the eggshell; these are secreted by the follicle cells specific to that area of the egg. Because of the ventralizing

effect of the *top*^{1} mutation, the number of appendage-secreting follicle cells is reduced, such that these two appendages become fused and only one dorsal appendage is apparent. The ventralized appendage is also positioned further back from the anterior of the egg, relative to the wild type. The ventralizing effect of *top*^{1} on the eggshell can also be seen in the embryo. In embryos that develop from eggs deposited by *top*^{1} mutant females, the amount of ventral tissue normally present is increased at the expense of dorsal tissues, in that cells which would otherwise commit to a dorsal fate assume a ventral fate. As the ventral mesoderm of the embryo is in excess, there is insufficient cuticle to surround the embryo, and so the embryo consists of an open strip of dorsal cuticle flanked by bands of ventral denticle material. The head structures of the embryo are also greatly reduced. These phenotypes are collectively referred to as the maternal effect embryonic lethal phenotype of *torpedo.*

While *torpedo* is playing an important role in the determination of dorsoventral polarity, there exist other genes, such as *gurken, cornichon, fs(1)K10, squid, cappuccino* and *spire*, which are also required in the female to correctly establish the dorsoventral patterning of the eggshell and embryo. As with *torpedo*, mutations in *gurken* and *cornichon* produce ventralized eggs and embryos (Schüpbach, 1987; Ashburner *et al.*, 1990). Females mutant for any of *fs(1)K10, squid, cappuccino* or *spire* produce eggshells and embryos whose phenotypes can be described as dorsalized (Wieschaus *et al.*, 1978; Wieschaus, 1979; Manseau and Schüpbach, 1989; Kelley, 1993). Extensive mosaic analysis has shown that the activities of *gurken, cornichon, fs(1)K10, squid, cappuccino* and *spire* are required in the germ line, while the activity of *torpedo* is required strictly in the somatically derived follicle cell epithelium. Genetic analysis has placed the four dorsalizing loci *fs(1)K10, squid, cappuccino* and *spire* upstream of the of the ventralizing loci *cornichon, gurken* and *torpedo* (Wieschaus *et al.*, 1978; Schüpbach, 1987; Manseau and Schüpbach, 1989; see Schüpbach *et al.*, 1991, for review). As the mutant phenotypes

of *gurken*, *cornichon* and *torpedo* are very similar, the mosaic and epistatic analyses suggest that these three genes are acting together in dorsoventral patterning, and that the wild type activities of *cornichon* and *gurken* are required to send a signal from the oocyte to the torpedo receptor on the membrane of the follicle cells facing the oocyte (see Shilo and Raz, 1991, for review); this signal would specify the dorsal fate.

The *gurken* locus was found to encode a TGFα-like molecule, and the *gurken* gene product is thought to serve as the ligand for the torpedo receptor during oogenesis, inducing the dorsal cell fate in the follicle cell epithelium (Neuman-Silberberg and Schüpbach, 1993, 1994). Transcripts from *gurken* normally accumulate near the dorsal-anterior cortex of the oocyte. Mutations in *fs(1)K10*, *squid*, *cappuccino* and *spire* result in the failure to localize the *gurken* transcript and lead to ubiquitous expression of the *gurken* signal, such that the resultant eggshells and embryos become dorsalized (Manseau and Schüpbach, 1989; Neuman-Silberberg and Schüpbach, 1993).

Members of the *spitz* gene group have been shown to play a role in dorsoventral patterning, as loss of *rhomboid* function in the egg chamber leads to the ventralization of the eggshell and the embryo (Ruohola-Baker *et al.*, 1993). It has also been shown that the activities of *gurken* and *torpedo* are required for the proper expression of *rhomboid* in the follicle cell epithelium. The *rhomboid* gene product is proposed to act as a limiting factor in the reception of the dorsal signal in that it may serve to facilitate the gurken-torpedo/ligand-receptor interactions (Ruohola-Baker *et al.*, 1993; Sturtevant *et al.*, 1993; Neuman-Silberberg and Schüpbach, 1994). The activity of *Star* is required for the normal development of the female germ line (Mayer and Nüsslein-Volhard, 1988), and mutations in *Star* have been found to increase the severity of the maternal effect embryonic lethal phenotype of *torpedo* (J. Price, unpublished results).

Current models of torpedo-mediated signaling in oogenesis place the *torpedo* gene product (EGF receptor) on the follicle cell membrane, where it receives a

dorsalizing signal (*gurken*) from the oocyte. The reception of this signal is perhaps modulated by rhomboid and Star. The activities of *fs(1)K10*, *squid*, *cappuccino* and *spire* appear to be restricting this signal to its appropriate location and ensuring that the dorsal fate is expressed only in the appropriate region of the egg chamber. The combined activities of all these seven genes results in the proper specification of the dorsoventral patterning of the eggshell and embryo.

***torpedo* is Required for the Development of the Compound Eye.**

The compound eye of *Drosophila* is composed of approximately 800 individual units called ommatidia. Each ommatidium is made up of eight photoreceptor cells (R1 through R8), four lens-secreting cone cells and eight accessory cells. The activity of *torpedo* is required for both the proliferation and differentiation of photoreceptor cells in the developing eye imaginal disc (Zak and Shilo, 1992; Xu and Rubin, 1993).

Mutations in *torpedo* are known to affect the development of the eye. A dominant, gain-of-function mutation of *torpedo*, called *Ellipse* (*Elp*), disrupts the organization of ommatidia as well as serving to reduce their number; the net result causes the eye to assume a roughened appearance (Baker and Rubin, 1989, 1992). Loss-of-function mutations of *torpedo*, such as top^1, also produce defects in the compound eye. The facets of the eye become irregular in shape, and occasionally fused, and the interommatidial bristles show an abnormal distribution, producing a rough eye phenotype (Clifford and Schüpbach, 1989; Price *et al.*, 1989).

Ellipse has shown genetic interaction with the neurogenic locus, *Notch*. The *split* mutation is a recessive allele of *Notch*, and imparts a rough eye phenotype; the *Ellipse* mutant behaves as a strong enhancer of the *split* phenotype (Baker and Rubin, 1992).

The *sevenless* receptor tyrosine kinase gene is known to be required exclusively for the development of the R7 photoreceptor cell (Banerjee *et al.*, 1987; Hafen *et al.*, 1987). A number of genes acting downstream of the sevenless receptor have been

identified based on their ability to modify the signaling activity of sevenless. In sequence, these genes are: *Downstream of receptor kinases* (*Drk*), *Son of sevenless* (*Sos*), *Ras1*, *raf/l(1)polehole*, *suppressor of raf* (*Dsor1*) and *rolled* (*rl*). The *Drk* locus encodes an SH3-SH2-SH3 adaptor protein which couples sevenless to Sos, a guanine nucleotide exchange factor for Ras1 (Rogge *et al.*, 1991; Simon *et al.*, 1991; Olivier *et al.*, 1993; Simon *et al.*, 1993). Ras1, in turn, activates raf, a MAPKK kinase (Ambrosio *et al.*, 1989; Dickson *et al.*, 1992). Downstream of raf is Dsor1, a MAPK kinase, which activates rolled, a MAP/mitogen-activated protein kinase (Howe *et al.*, 1992; Tsuda *et al.*, 1993; Biggs *et al.*, 1994).

The downstream members of the sevenless pathway are known to interact with the *torpedo* locus. Genetic analysis has shown that dominant, loss-of-function mutations in *Drk*, *Sos*, *Ras1*, and an *Enhancer of sevenless* mutation known as *E(sev)1A* are capable of suppressing the rough eye phenotype of the *Ellipse* mutation (Simon *et al.*, 1991), while a gain of function *Sos* mutation was shown to enhance the *Ellipse* phenotype (Rogge *et al.*, 1991). Gain-of function mutations in both *raf* and *Dsor1* have been found to act as potent suppressors of the *faint little ball* embryonic lethal phenotype of *torpedo* (Tsuda *et al.*, 1993). A gain-of-function mutation in *rolled* has been demonstrated to strongly enhance the roughness of the *Ellipse* eye (Brunner *et al.*, 1994).

The *Drosophila* gene *torso* encodes another receptor tyrosine kinase, and is required for the determination of anterior and posterior terminal structures - the acron and the telson - in the embryo (Klingler *et al.*, 1988; Sprenger *et al.*, 1989). The torso signaling pathway has been shown to share downstream components with both the sevenless and torpedo pathways. After conducting a screen for dominant suppressors of a gain-of-function mutation in *torso*, two of these *Su(tor)* mutations were also found to be capable of suppressing the *Ellipse* phenotype. These two mutations were determined to be allelic to *Ras1* and *Sos* (Doyle and Bishop, 1993), which were previously described as

dominant *Enhancers of sevenless* (Simon *et al.*, 1991). In further attempts to describe interactions between components of the torso and sevenless pathways, it was observed that the *E(sev)1A* mutation reported by Simon *et al.* (1991) also displayed *Su(tor)* activity. The *E(sev)1A* mutation was shown to be allelic to the *corkscrew* locus, a putative protein tyrosine phosphatase (PTPase) which functions to transduce the terminal signal from the torso receptor to downstream components of the torso pathway (Perkins *et al.*, 1992; Doyle and Bishop, 1993).

Star is a gene which plays an essential role in the early events of ommatidial cluster formation, as the activity of *Star* is required for the development of the R2, R5 and R8 photoreceptor cells. Dominant, loss-of-function mutations of the *Star* locus produce a rough eye phenotype (Heberlein and Rubin, 1991). *Star* has also demonstrated interactions with members of the sevenless pathway. *Star* and *Ras1* mutants act synergistically to produce a severely reduced and roughened eye, as does the combination of the *Star* and *E(sev)1A/corkscrew* mutations. *Star* and *Sos* also act together to produce an increased roughness of the eye, as well as a slight reduction in its size (Heberlein *et al.*, 1993). As sevenless signalling components are known to interact with *torpedo*, it is interesting to note that *Star* also interacts with the *torpedo* locus. *Star* and *Ellipse* mutations act synergistically to produce a very small and very rough eye, a phenotype more severe than either *Star* or *Ellipse* can produce on its own (Kolodkin *et al.*, 1994). *Star* has also been shown to enhance the eye defects of the recessive *torpedo* mutation, top^1 (J. Price, unpublished results).

***torpedo* is Required for Wing Vein Pattern Formation.**

Mutations in *torpedo* also affect the patterning of the wing veins. The *Ellipse* gain-of-function mutation has been shown to result in ectopic wing vein formation, as seen by the presence of wing vein branchings (Baker and Rubin, 1992). The recessive top^1 mutation causes the deletion of the anterior cross vein and, in combination with

other recessive alleles of *torpedo*, is capable of producing gaps in the fourth longitudinal (L4) wing vein (Clifford and Schüpbach, 1989; Price *et al.*, 1989).

The members of the sevenless signaling cassette are exclusively required for torpedo-mediated signaling in the development of epidermal tissues, as loss-of-function mutations in any of *Drk*, *Sos*, *Ras1*, *raf* and *rolled* have been shown to produce wing vein phenotypes similar to those seen with hypomorphic mutations in *torpedo* (Diaz-Benjumea and Hafen, 1994). A loss-of-function *rolled* mutant shows a very strong enhancement of the top^1 wing vein defects, deleting a large amount of wing vein material. A gain-of-function mutation in the *rolled* locus also showed strong enhancement of the ectopic wing vein phenotype of the *Ellipse* mutation of *torpedo* (Diaz-Benjumea and Hafen, 1994; Brunner *et al.*, 1994).

The *torpedo* locus also exhibits genetic interaction with three of the neurogenic loci, *Notch*, *Delta* and *Hairless*. The *Ellipse* mutation has been shown to enhance the wing-notching phenotype of *Notch*, and behaves as a particularly strong enhancer of the ectopic wing vein phenotype of *Delta* (Baker and Rubin, 1992). *Hairless* is also known to interact with *torpedo*, as seen by the ability of hypomorphic *Hairless* mutants to increase the severity of the wing vein defects associated with the top^1 allele (J. Price, unpublished results; this work, Figure 6).

At least two members of the *spitz* gene group are known to be required for the development of the wing. The *rhomboid* gene also plays a part in the establishment of wing vein patterns, as flies homozygous for loss-of-function mutations in *rhomboid* are missing the fourth and fifth longitudinal wing veins. The *rhomboid* locus also displays genetic interaction with *torpedo* in the wing. Wings double-mutant for hypomorphic alleles of *torpedo* and *rhomboid* show a significant enhancement of *rhomboid* wing vein defects, while the *Ellipse* mutation of *torpedo* is able to almost completely suppress the *rhomboid* phenotype (Sturtevant *et al.*, 1993). The activity of *Star* is also required for

wing vein formation, as clones of homozygous *Star* mutants cause the shortening of the second and third longitudinal wing veins. *Star* is also known to interact with *torpedo* in the wing, as dominant, loss-of-function mutations in *Star* are capable of suppressing the ectopic wing vein phenotype of *Ellipse* (Heberlein *et al.*, 1993). *Star* mutants have also been shown to enhance the wing vein defects of the top^1 mutation (J. Price, unpublished results; this work, Figure 6).

The activity of the *torpedo* locus is required in a great variety of developmental processes and displays genetic interactions with a great host of other genes. Given this fact, it is of considerable interest to identify additional loci which interact with *torpedo*. Hypomorphic mutations in *Hairless* and *Star*, both known to interact with *torpedo*, are able to produce L4 gaps in a top^1 mutant background (J. Price, unpublished results; this work, Figure 6), a phenotype which cannot be attributed to the top^1 mutation. With this in mind, it should be possible to conduct a genetic screen for dominant mutations in loci which interact with *torpedo*. Any mutation capable of producing L4 defects in a top^1 mutant wing would be classified an *Enhancer of torpedo*, or *E(top)*.

Four screens of this nature were carried out, and a total of 25 *E(top)* mutations were recovered. Twenty of these mutations were mapped to the second chromosome; five were mapped to the third chromosome. David Lum, who conducted two of the four screens, began the preliminary characterizations of the 20 second-chromosomal *E(top)*s. This work consists of a more detailed genetic and phenotypic analysis of all 25 *E(top)* mutations.

The *E(top)*s of the second chromosome were subjected to extensive genetic and phenotypic characterization. The exact number of loci represented by these 20 mutations has not yet been established. However, preliminary results indicate that at least one locus distinct from *torpedo* has been identified.

Greater attention has been given to the five *E(top)*s of the third chromosome. Genetic and phenotypic data are consistent with the notion that these five mutations each represent a single locus. Results have been obtained which show that three of these *E(top)*s behave as dominant enhancers of another gene, called *Hairless*. Map positions are also reported for these three mutations.

Materials and Methods.

Fly Food.

All fly stocks and all crosses were raised on corn meal agar medium prepared according to Cline (1978).

The Screens for Dominant *Enhancers of torpedo*.

These screens were designed to generate dominant *Enhancers of torpedo* on the second and third chromosomes. Male flies of genotype *b pr cn top*^{1} *bw / b pr cn top*^{1} *bw* were treated with 0.05 M EMS in 1% sucrose for 24 hours. These mutagenized males were then mated to virgin females of genotype *top*^{1} *bw / CyO DTS-100*. The females were allowed to lay eggs for 5 days and then transferred to fresh media. The eggs and developing embryos were then shifted to 29° C, the restrictive temperature for the *CyO DTS-100* chromosome. Under these conditions, only animals of genotype *b pr cn top*^{1} *bw* / + + + *top*^{1} *bw* + ; + / + would survive to adulthood. Adult F_1 males of genotype *(b pr cn top*^{1} *bw)** / + + + *top*^{1} *bw* + ; *(+)** / + were examined phenotypically for defects in the fourth longitudinal wing vein (L4). Males with L4 defects were retained as putative carriers of novel *Enhancers of torpedo* [*E(top)*s]. These flies were then crossed to virgin females of genotype *top*^{1} *bw / CyO*, and male progeny of genotype *b pr cn top*^{1} *bw E(top)*? / + + + *top*^{1} *bw ; E(top)*? / + were again selected for the characteristic L4 defects to ensure the original enhancement seen was, in fact, due to a heritable trait, and not to some transient effect(s). Those mutations which successfully passed this rescreening - in other words, those flies which did, indeed, carry a putative *Enhancer of torpedo* - were then assigned to their particular chromosomes. As the aim of the screen was to find putative *E(top)*s on the second and third chromosomes, individual males displaying the *E(top)* phenotype were pair-mated to a virgin female of genotype *b pr cn top*^{1} *bw / CyO*. *E(top)* phenotypes which segregated exclusively with the *b pr cn top*^{1} *bw* chromosome were assigned to chromosome 2, while those defects which segregated equally with both the *b pr cn top*^{1} *bw* and *top*^{1} *bw* chromosomes were

assigned to chromosome 3.

Four screens were conducted in total: one screen by J. Price, two screens by David H. Lum, and a similar screen by Karen Ann Rempel. J. Price's screen produced four *E(top)* mutations on the third chromosome, *E(top)s 2-2*, *2-21*, *C1-2* and H^{21C}, recovered from approximately 2,000 chromosomes. The H^{21C} mutation was shown to be an allele of the *Hairless* gene. David Lum's screen yielded 20 *E(top)*s on the second chromosome and three *E(top)*s on the third chromosome [*E(top) D105* and two alleles of *Hairless*]. David Lum tested roughly 22,000 chromosomes in two screens. Karen Ann Rempel isolated one mutation on the third chromosome, *E(top) K24* (renamed *Bare naked fly*; see below), recovered from approximately 750 chromosomes tested. See Table 1 for a listing of all the mutations characterized in this thesis.

Egg Collections/Embryo Preparation.

Apple juice agar medium plates were used in all egg collections. Eggshells were removed by treating the eggs with sodium hypochlorite (common household bleach). Embryos were fixed in a 1:1 solution of glacial acetic acid and glycerol, then heated for approximately 15 minutes at 60° C. The fixed embryos were placed in Hoyer's mountant and then heated for approximatley three hours at 60° C. All media were prepared and all procedures were carried out in accordance with Wieschaus and Nüsslein-Volhard (1986).

Genetic Mapping of the Third-Chromosomal *Enhancers of torpedo*.

The *ru cu ca* and *ru Pr ca* chromosomes were used for the mapping of the *E(top)*s on the third chromosome. The complete genetic makeup of these chromosomes is as follows: *ru cu ca* is equivalent to *ru h th st cu sr e ca*, where *ru* = *roughoid* (0.0), *h* = *hairy* (26.5), *th* = *thread* (43.2), *st* = *scarlet* (44.0), *cu* = *curled* (50.0), *sr* = *stripe* (62.0), *e* = *ebony* (70.7), and *ca* = *claret* (100.7). Numbers appearing after the gene indicate the genetic position of the marker on chromosome 3. The *ru Pr ca* chromosome has the same genetic construct as *ru cu ca*, except *ru Pr ca* also carries the

dominant mutation *Pr* = *Prickly* (90.0), which lies between the markers *ebony* and *claret*.

Males of genotype *E(top) / TM3* were mated to *ru cu ca / TM3* virgin females. Heterozygous virgin females of genotype *E(top) / ru cu ca* were selected and were then mated to *ru Pr ca / TM3* males. The F_1 recombinant male progeny of genotype *E(top) recomb. / ru Pr ca* were then scored for the phenotypic markers of the *ru cu ca* chromosome. In the cases of *E(top)*s *2-21*, *D105* and *K24*, it was found that these *E(top)* chromosomes also displayed *Enhancer of Hairless*, or *E(H)* activity. The *E(H)* phenotype was mapped to determine if the *E(top)* and *E(H)* activities could be separated by recombination. Each F_1 male was pair-mated to a H^{21C} / *TM3* virgin female. The *E(top) recomb.* / H^{21C} progeny were scored for enhancement of the *Hairless* phenotype by the recombinant chromosome.

Wing Mounts.

Wings were dissected from anaesthetized flies and placed in ethanol. The wings were then removed wet from the ethanol and placed into several drops of Permount histological mounting medium on a flat microscope slide. A cover slip was placed on top of the wings and the slide was set aside and allowed to dry.

Photography.

All photographs of wing and bristle patterns were taken using the Wild MPS 45/51 Photoautomat camera apparatus by Wild Heerbrugg Ltd. The photographs in Figures 1 and 2 were taken under phase contrast illumination on the Olympus Vanox AHBS3 microscope, using a 10X phase contrast lens and a 2.5X Optivar photolens. The film used in all photographs was TMAX Professional film (100 ASA, for black-and-white prints) by Kodak Canada, Inc.

Table 1.

Mutations Characterized in this Thesis.

Mutation	Source	Reference
top[1]	Schüpbach	Schüpbach, 1987
E(top) D3	David H. Lum	unpublished
E(top) D9	David H. Lum	unpublished
E(top) D20	David H. Lum	unpublished
E(top) D29	David H. Lum	unpublished
E(top) D30	David H. Lum	unpublished
E(top) D43	David H. Lum	unpublished
E(top) D50	David H. Lum	unpublished
E(top) D56	David H. Lum	unpublished
E(top) D59	David H. Lum	unpublished
E(top) D67	David H. Lum	unpublished
E(top) D76	David H. Lum	unpublished
E(top) D81	David H. Lum	unpublished
E(top) D82	David H. Lum	unpublished
E(top) D84	David H. Lum	unpublished
E(top) D85	David H. Lum	unpublished
E(top) D86	David H. Lum	unpublished
E(top) D106	David H. Lum	unpublished
E(top) D112	David H. Lum	unpublished
E(top) 2-2	James V. Price	unpublished

Table 1. (continued)

Mutations Characterized in this Thesis.

Mutation	Source	Reference
E(top) 2-21	James V. Price	unpublished
E(top) C1-2	James V. Price	unpublished
H^{21C}	James V. Price	unpublished
E(top) D57 (H^{D57})	David H. Lum	unpublished
E(top) D73 (H^{D73})	David H. Lum	unpublished
E(top) D105	David H. Lum	unpublished
E(top) K24 (Bnf)	Karen Ann Rempel	unpublished

Results.

Section I.

The *Enhancers of torpedo* of the Second Chromosome.

Four screens for dominant *Enhancers of torpedo*, or *E(top)*s, were performed (see **Materials and Methods**). From these screens, a total of 25 dominant *E(top)*s were recovered. Twenty were mapped to the second chromosome; five were mapped to the third chromosome. In this section, the analysis of the *E(top)*s of the second chromosome is presented.

Male flies of genotype *b pr cn top*^{1} *bw E(top) / CyO* were mated individually to *top*^{1} *bw / CyO* virgin females. From amongst the F_1 progeny, male and female flies of genotype *b pr cn top*^{1} *bw E(top) / + + + top*^{1} *bw* + were selected. These flies were examined phenotypically in order to assess the ability of each mutation to enhance the phenotypes associated with the *top*^{1} allele. The following served as criteria to ascertain the degree of enhancement: the ventralization of the eggshell as well as the ventralization of the embryo (the maternal effect embryonic lethal phenotype), the roughening of the compound eye, and the presence of gaps in the fourth longitudinal (L4) wing vein. While mutations in *torpedo* are known to cause deletions of certain sensory bristles from the head and thorax (Clifford and Schüpbach, 1989; Price *et al.*, 1989), this aspect of the *torpedo* phenotype was not used as part of the assessment of the *E(top)* phenotype of the second-chromosomal mutations. In addition, the phenotypes of those *E(top)*s which behaved as embryonic lethals were documented. The chromosome 2 *E(top)*s were also subjected to three different complementation analyses.

The Maternal Effect Phenotypes.

Females homozygous for hypomorphic mutations in *torpedo*, such as the *top*^{1} allele, lay eggs in which both the embryo and the eggshell (chorion) are ventralized. In a wild type eggshell, two structures are apparent on the dorsoanterior region of the egg. These structures, referred to as dorsal appendages, are secreted by the follicle cells as part of the mature eggshell. In an egg laid by a *top*^{1} homozygous female, these dorsal appendages become fused, such that only one dorsal appendage is apparent (Schüpbach,

1987).

Virgin females, homozygous for *top*^{1} and heterozygous for an *Enhancer of torpedo* mutation - genotype: *b pr cn top*^{1} *bw E(top) / + + + top*^{1} *bw* + - were mated to wild type (*ORE R*) males and allowed to lay eggs. The eggs were then collected and examined for any increase in the ventralization of the chorion.

Eggs deposited by *top*^{1} females carrying the *E(top)* mutations *D3*, *D9*, *D20*, *D43*, *D44*, *D56*, *D59*, *D69*, *D81*, *D84*, *D85*, *D86*, *D106* and *D112* showed only a slight reduction in the amount of dorsal appendage material present. Their enhancement of the eggshell's morphology was considered to be weak. The *D29*, *D30*, *D50*, *D67* and *D76* mutations produced a moderate increase in the severity of eggshell defects in eggs laid by females mutant for *top*^{1}. Some of the eggs' chorions were ventralized to such an extent that there was essentially no dorsal appendage material, save for a small stump of the dorsal appendage which was positioned further back from the anterior of the egg (relative to *top*^{1} chorions). While the eggshell phenotype associated with the *D30* mutation was only moderate with respect to its increase in ventralization, the vitelline membrane of these eggs was affected such that it appeared to be structurally very weak. None of the eggs collected from *top*^{1} females carrying the *D30* mutation survived preparation procedures, as the vitelline membrane was ruptured in all cases. In *top*^{1} mutant females, the *D82* mutation showed the strongest enhancement of *top*^{1} defects, relative to the other 19 *E(top)*s of the second chromosome. These females produced the greatest proportion of eggs which showed a reduction in the amount of dorsal appendage material. Where appendages are visible, some showed only a residual stump of appendage material. Approximately 15% of the eggs had no dorsal appendages whatsoever, and these eggs were more elongated and narrower with respect to *top*^{1} eggs. Another defect was also observed in some of these eggs, in that the micropyle was greatly reduced relative to the wild type. In one case, the micropyle was entirely absent (Figure 2).

Figure 2.

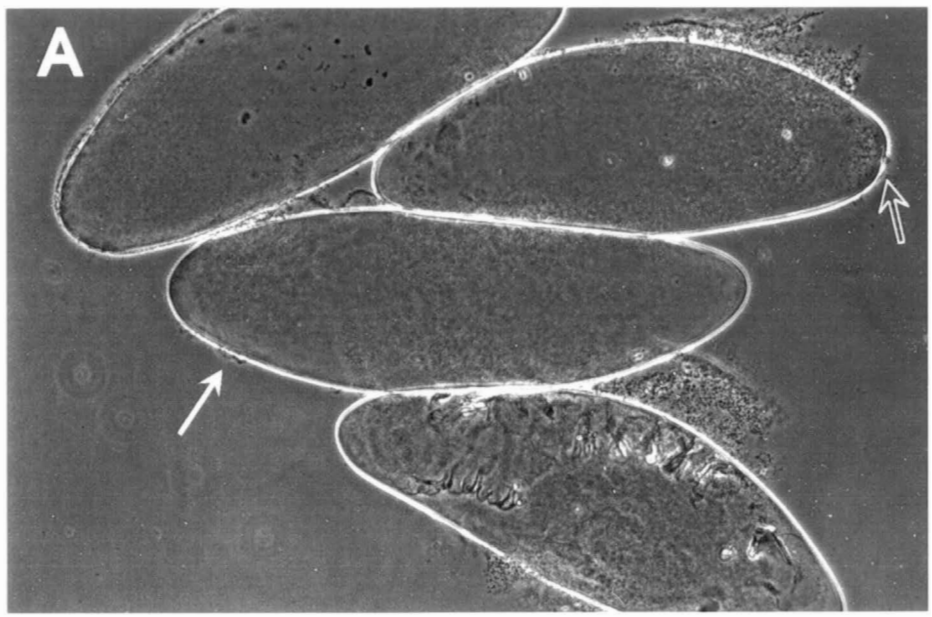
Enhancement of *top*^{1} Egg Morphology by *E(top) D82*.

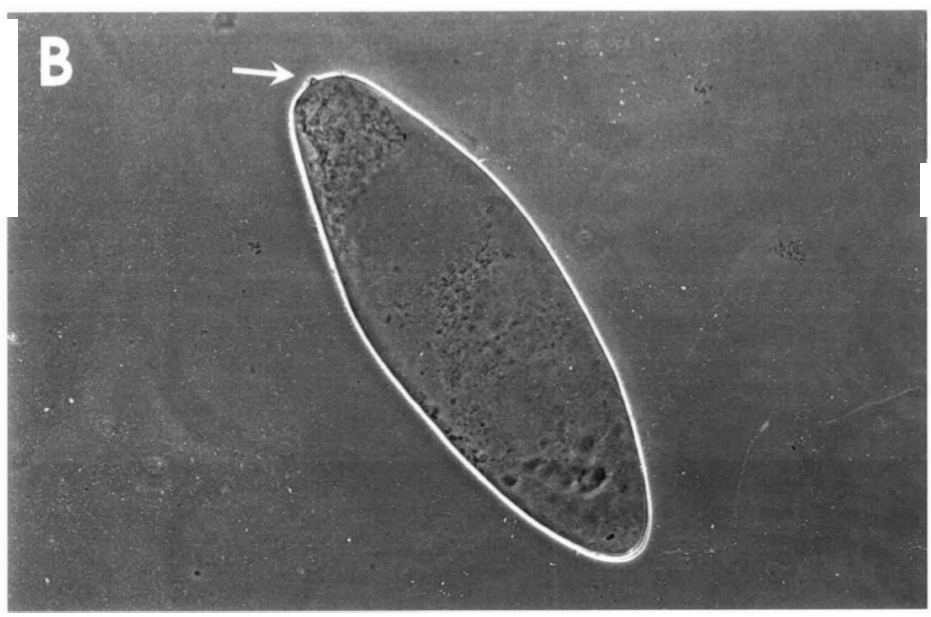
The eggs in both (A) and (B) were collected from females of the following genotype: *b pr cn top*^{1} *bw E(top) D82 / + + + top*^{1} *bw +*.

(A) shows two eggs of interest. The egg at centre (solid arrow) has no micropyle. The egg in the upper right of the photograph has a reduced micropyle, as indicated by the hollow arrow.

(B) shows another egg collected from the same females. The micropyle of this egg (as indicated by the arrow) is also very much reduced. Compare the micropyle of this egg to the wild type micropyle of the egg in Figure 3F (hollow arrow).

None of the eggs which displayed defects in the micropyle were fertilized. No further eggs could be obtained from the females of the above genotype.


A


B

The second-chromosomal *E(top)*s were also examined for their ability to enhance the maternal effect embryonic lethality associated with the *top*^{1} allele. In wild type embryos, a distinct series of ventrally located denticle bands is apparent. In an egg laid by a female mutant for *top*^{1}, the dorsal cuticle is flanked by these denticle belts, but the amount of cuticle present is insufficient to encompass the embryo as there is an excess of ventral mesoderm. The embryo thus appears as an open sack of cuticle. The head structures of the embryo are also strongly disrupted (Schüpbach, 1987). Embryos were collected from the same *b pr cn top*^{1} *bw E(top) / + + + top*^{1} *bw +* females as above, and were examined for any enhancement of these maternal effect embryonic lethal phenotypes. The head and ventral cuticle defects were used as criteria in assessing the degree of phenotypic enhancement. While the defects in head structures appeared only slightly more severe, and while the embryos seemed somewhat shorter in overall length, no significant enhancement of the *top*^{1} maternal effect embryonic lethal phenotypes was observed. Consequently, the second-chromosomal *E(top)*s' ability to enhance these phenotypes was considered to be weak. The ability of the *E(top) D30* mutation to enhance the maternal effect lethal phenotype of the embryo could not be determined, as none of these females' eggs were fertilized. For a summary of the maternal effect phenotypes associated with the *E(top)*s of the second chromosome, refer to Table 2, Columns 1 and 2.

As *top*^{1} females heterozygous for one of *E(top)*s *D20*, *D29*, *D30*, *D67*, *D76*, *D82* and *D85* laid very few eggs, attempts were made to obtain more. Females of these genetic constructs did not lay any eggs in repeat experiments. Thus, these mutant strains showed reduced fecundity when in combination with *top*^{1}.

Six mutations, *E(top)*s *D3*, *D43*, *D59*, *D81*, *D84* and *D112*, were found to be viable when homozygous, and it was necessary to determine if these mutations were able to enhance the maternal effect phenotypes to any significant degree when in the

homozygous condition. Homozygous *b pr cn top*^{1} *bw E(top)* virgin females were mated to *ORE R* males, and then their eggs were collected and subjected to the same phenotypic examinations as above. Females homozygous for *top*^{1} and one of *E(top)*s *D3*, *D43*, *D81*, *D84* and *D112* laid eggs whose chorions showed a moderate increase in ventralization over females heterozygous for the same *E(top)* mutation. Eggs from females homozygous for *E(top) D59* showed no increase in ventralization over the eggs laid by *E(top) D59* heterozygous females. As homozygotes, none of these six mutations showed any ability to enhance the maternal effect embryonic lethal phenotype (above phenotypes not shown).

Eye Phenotypes.

Mutations in *torpedo* are known to produce defects in the compound eye of the adult fly. In *top*^{1} mutants, the eye's facets are irregularly shaped and occasionally fused. Rows of ommatidia are disorganized and the bristles between the ommatidia show an abnormal distribution. The result of these combined defects is to cause the eye to take on a roughened appearance (Clifford and Schüpbach, 1989; Price *et al.*, 1989).

The flies of genotype *b pr cn top*^{1} *bw E(top)* / + + + *top*^{1} *bw* + were examined for enhancement of the rough eye phenotype associated with the *top*^{1} mutation. *E(top)*s *D3*, *D9*, *D43*, *D56*, *D59*, *D81*, *D84*, *D106* and *D112* did not show any enhancement of the eye's roughness. *E(top) D50* showed only a slight increase in the severity of the *top*^{1} eye phenotype. The *E(top)* mutations *D20*, *D29*, *D30*, *D44*, *D67*, *D69*, *D76*, *D82*, *D85* and *D86* behaved as very strong enhancers of the eye's roughness. The eyes of these flies were smaller and somewhat narrower than seen in flies mutant only for *top*^{1}. The number of ommatidia was very much reduced, and the organization of both the ommatidia and the interommatidial bristles was worsened, relative to *top*^{1} eyes. A greater number of ommatidia also appeared to be fused in the eyes of flies which carried these mutations. For a summary of eye phenotypes produced by these mutations, see Table 2, Column 3.

Those *E(top)*s which were viable as homozygotes were not found to increase the severity of the top^1 eye defects (phenotypes not shown).

Wing Phenotypes.

Mutations in *torpedo* are also known to cause disruptions in the venation pattern of the adult wing. Flies mutant for top^1 display a subtle phenotype, as seen by the deletion of the anterior cross vein (Figure 4B). Stronger mutations in *torpedo* are capable of producing gaps in the fourth longitudinal vein (Clifford and Schüpbach, 1989; Price *et al.*, 1989; Figure 4, G and H). All of the *E(top)*s isolated in the screens were recovered by specifically selecting for mutations which produced defects in the fourth longitudinal vein (L4).

While all 20 *E(top)*s initially displayed L4 defects, this effect diminished with time. At the time this thesis was written, the *E(top)* mutations *D3*, *D9*, *D84* and *D112* no longer showed any disruption of the L4 vein. *E(top)*s *D43*, *D44*, *D50*, *D56*, *D59*, *D76* and *D81* showed a certain degree of variability in the amount of the L4 vein which was deleted. The most extreme L4 gaps seen could extend from the margin of the wing to a point proximal to the posterior cross vein (PCV). The minimum perturbation that was scored as an L4 defect corresponded to the deletion of a small portion of the wing vein from the segment between the PCV and the wing's margin. The *E(top)* mutations *D20*, *D29*, *D30*, *D67*, *D69*, *D82*, *D85*, and *D86* were consistent in their ability to remove a segment of the L4 vein which stretched from the PCV to a point close to the margin of the wing. *E(top)*s *D20*, *D30* and *D82* disrupted wing veins other than L4. All three of these mutations produced small deletions in the third longitudinal vein (L3). *E(top) D82* was also able to delete a small segment from the second longitudinal vein (L2). These wing vein phenotypes are considered significant, as no such phenotype has been seen in association with previously described *E(top)* phenotypes.

With the exception of the *E(top) D3*, *D9*, *D84* and *D112* mutations, all other

Table 2.

Summary of Phenotypes for the *Enhancers of torpedo* of the Second Chromosome.

Table 2 shows a summary of the phenotypes produced by the *E(top)*s of the second chromosome. All phenotypes are for *b pr cn top*^{1} *bw E(top)* / + + + *top*^{1} *bw* + flies, unless otherwise noted. Only females were assessed for maternal effect phenotypes (Columns 1 and 2); both males and females were scored for the adult phenotypes (eyes and wings; Columns 3 through 5).

(1) MEEL = maternal effect embryonic lethal.

(2) L2, 3 and 4 = second, third and fourth longitudinal wing veins, respectively; PCV = posterior cross vein.

(3) Deficiency of *torpedo* used was *Df(2R)top*^{18A} (Price et al., 1989).

(4) Chorions of eggs laid by *b pr cn top*^{1} *bw E(top)* homozygous females showed a moderate increase in ventralization.

(5) These mutations showed very low fecundity in their respective genetic constructs; very few eggs were obtained in initial egg collections, and no eggs were obtained in repeat experiments.

(6) N/D = Not determined. The enhancement of the MEEL phenotype could not be assessed, as none of the eggs laid by these females were fertilized.

(7) These mutations initially showed disruptions of the L4 wing vein; these defects are no longer seen. See text for discussion.

(8) *E(top)*s *D3* and *D84* showed L4 and PCV defects only as *b pr cn top*^{1} *bw E(top)* homozygotes.

	Enhancement of torpedo egg morphology	Enhancement of torpedo MEEL phenotype (1)	Enhancement of torpedo eye defects	Disruption of the L4 wing vein	Disruption of the L3 wing vein	Disruption of the L2 wing vein	Disruption of the PCV (2)	Viable in the homozygous condition	Viable over a Deficiency of torpedo (3)
E(top)									
D3	weak (4)	weak	none	none (7,8)	none	none	none (8)	yes	yes
D43	weak (4)	weak	none	variable	none	none	partial	yes	yes
D59	weak	weak	none	variable	none	none	partial	yes	yes
D81	weak (4)	weak	none	variable	none	none	partial	yes	yes
D84	weak (4)	weak	none	none (7,8)	none	none	none (8)	yes	yes
D112	weak (4)	weak	none	none (7)	none	none	none	yes	yes
D9	weak	weak	none	none (7)	none	none	none	no	yes
D56	weak	weak	none	variable	none	none	partial	no	yes
D106	weak	weak	none	partial	none	none	partial	no	yes
D20	weak (5)	weak	strong	partial	variable	none	partial	no	no
D29	moderate (5)	weak	strong	partial	none	none	partial	no	no
D30	moderate (5)	N/D (6)	strong	partial	variable	none	partial	no	no
D44	weak	weak	strong	variable	none	none	partial	no	no
D50	moderate	weak	weak	variable	none	none	partial	no	no
D67	moderate (5)	weak	strong	partial	none	none	partial	no	no
D69	weak	weak	strong	partial	none	none	partial	no	no
D76	moderate (5)	weak	strong	variable	none	none	partial	no	no
D82	strong (5)	weak	strong	partial	variable	variable	partial	no	no
D85	weak (5)	weak	strong	partial	none	none	partial	no	no
D86	weak	weak	strong	partial	none	none	partial	no	no

*E(top)*s showed defects in the PCV. These defects were seen as either the deletion of the anterior portion of the vein or as a merging of the anterior portion of the PCV and the proximal segment of the fourth longitudinal vein, such that the L4 vein appeared to curve downwards into the PCV (this was frequently seen in association with a gap in the L4 vein). Such observations were recorded as partial disruptions of the PCV. For a summary of the above wing vein defects, see Table 2, Columns 4 through 7.

Of the *E(top)*s that were homozygous viable, *D3*, *D43*, *D59*, *D81*, *D84* and *D112*, only the *E(top) D3* and *E(top) D84* homozygotes showed any increased severity of wing vein defects over their heterozygous counterparts. As heterozygotes, *E(top)*s *D3* and *D84* showed no defects in either the L4 vein or the PCV; as homozygotes, both mutations produced partial disruptions in these wing veins (phenotypes not shown).

Embryonic Lethal Phenotypes.

*E(top)*s *D20*, *D29*, *D30*, *D44*, *D50*, *D67*, *D69*, *D76*, *D82*, *D85* and *D86* all behave as embryonic lethals when homozygous. Embryos were collected as eggs which failed to hatch; these embryos were assessed for any defects in the cuticle. The embryos, which were presumed to be *b pr cn top*^{1} *bw E(top)* homozygotes, all displayed similar phenotypes. The embryos consisted of a naked layer of epidermis, with no sign of ventral denticle bands or dorsal hairs having formed. Their head cuticles were greatly disrupted and poorly organized, and were barely visible in some embryos. These phenotypes are similar to those seen with weak or moderate embryonic lethal phenotypes of *torpedo*, known as *faint little ball*. Embryonic lethal phenotypes are shown in Figure 3.

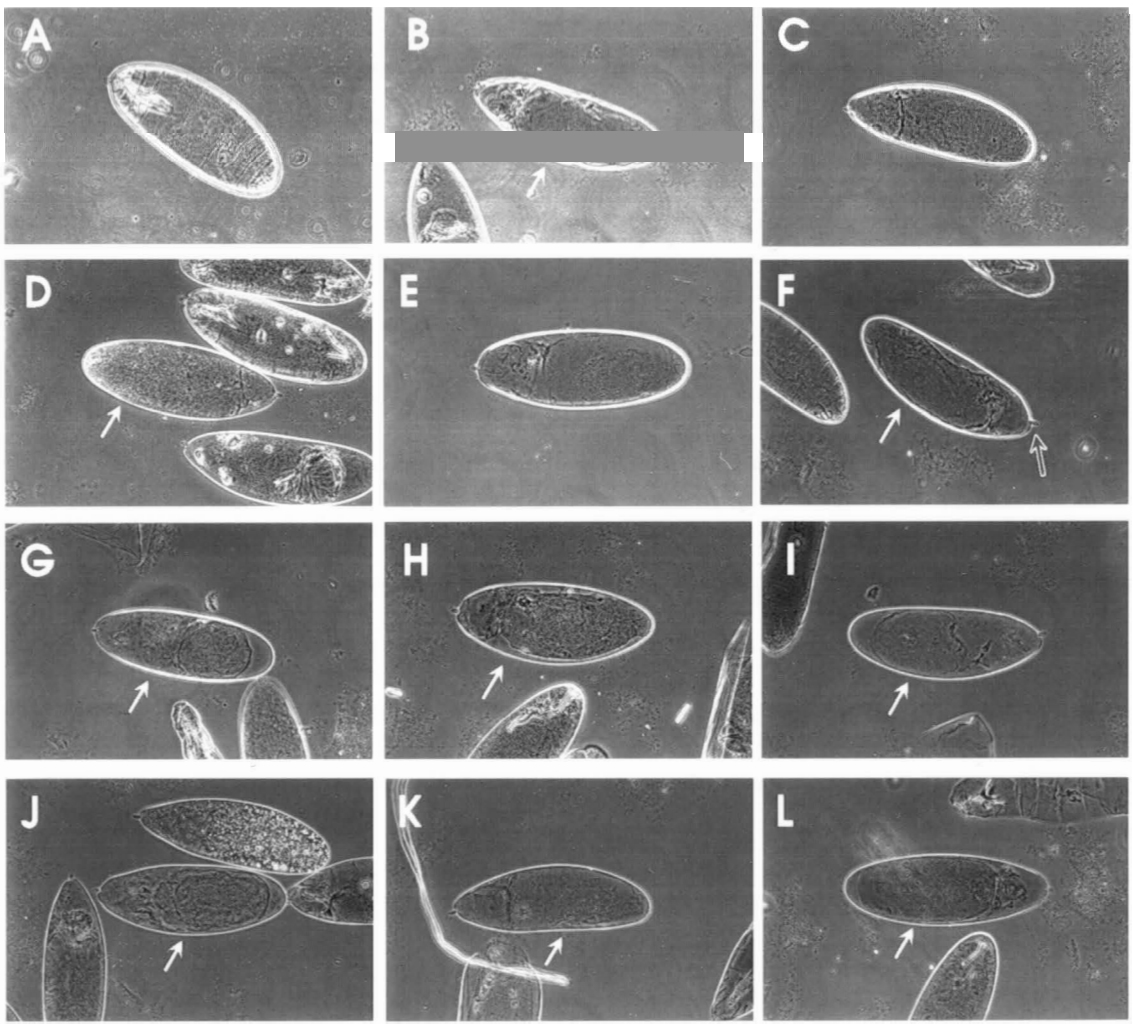
While *E(top)*s *D9*, *D56* and *D106* were also found to be embryonic lethals, no obvious cuticle defects were observed in these embryos (phenotypes not shown). This lethality may be due to independent mutations induced during the screen, at other locations on the chromosome.

Figure 3.

Embryonic Lethal Phenotypes of a Group of Second-Chromosomal *Enhancers of torpedo*.

In photographs (B) through (L), the embryos shown were collected as unhatched eggs from a cross between males and females of genotype *b pr cn top*^{1} *bw E(top) / CyO*. The embryos are presumably *b pr cn top*^{1} *bw E(top)* homozygotes; for individual photographs, only the mutations' designations shall be given. All embryos show a similar phenotype; the severely disrupted head cuticle and a complete lack of both dorsal hairs and ventral denticle bands are typical for this collection of *E(top)* mutations. Note that in those photographs where solid arrows are present they indicate the egg of interest.

(A) shows a wild type (*ORE R*) embryo, showing a well developed head cuticle and the organized rows of ventrally located denticle bands. (B), an *E(top) D20* homozygote. (C), an *E(top) D29* homozygote. (D), an *E(top) D30* homozygote; the head cuticle is very faint. In the lower right of the photograph, an embryo can be seen which is quite similar to a severe *faint little ball* embryo (the embryonic lethal phenotype of *torpedo*). (E), an *E(top) D44* homozygote. (F), an embryo homozygous for the *E(top) D50* mutation. The solid arrow indicates the embryo of interest; the hollow arrow is in reference to the normally formed micropyle (compare to the micropyles shown in Figure 2). (G), an *E(top) D67* homomzygote. The embryo is somewhat contracted in overall length and is similar to a moderate *faint little ball* embryo. (H), a homozygous *E(top) D69* embryo. (I) shows as *E(top) D76* homozygote. The embryo is fairly contracted with respect to its length, and is similar to a moderate *faint little ball* phenotype. (J), an *E(top) D82* homozygote, similar to a weak *faint little ball* embryo. (K), an *E(top) D85* homozygote. (L), an *E(top) D86* homozygote.


A
B
C
D
E
F
G
H
I
J
K
L

Complementation Analyses.

The chromosome 2 *E(top)*s were first examined for viability when homozygous by looking for *b pr cn top*^{1} *bw E(top)* homozygotes within every enhancer strain. The *E(top) D3*, *D43*, *D59*, *D81*, *D84* and *D112* chromosomes were homozygous viable. All others, *D9*, *D20*, *D29*, *D30*, *D44*, *D50*, *D56*, *D67*, *D69*, *D76*, *D82*, *D85*, *D86* and *D106*, were lethal as homozygotes (see Table 2, Column 8; Table 3). Three different series of complementation tests were carried out in the genetic characterization of these mutations. The *E(top)*s were tested against a null allele of *torpedo*, an allele of the *Star* locus, and were then tested against each other in *inter se* complementation tests.

When combined with *top*^{1}, strong alleles of the *torpedo* locus can produce eye and wing vein defects in the adult fly quite similar to those seen with the *E(top)*s of the second chromosome (Clifford and Schüpbach, 1989; Price *et al.*, 1989; J. Price, personal communication). Since the screening protocols used in the identification of putative *E(top)*s selected for phenotypes expected for strong alleles of *torpedo*, it was necessary to determine if any of the putative *E(top)*s recovered in the screen were actually strong alleles of *torpedo*. An allele of *torpedo* which behaved as a complete null, *top*^{18A}, was used to test for complementation of embryonic lethality associated with strong alleles of *torpedo*. The *top*^{18A} allele is a deficiency, *Df(2R)top*^{18A}, which removes only the *torpedo* locus (Price *et al.*, 1989). Flies of genotype *b pr cn top*^{1} *bw E(top) / CyO* were mated individually to flies carrying the *top*^{18A} deficiency. The *E(top)* mutations *D3*, *D43*, *D59*, *D81*, *D84*, *D112*, *D9*, *D56* and *D106* were all viable over the deficiency of *torpedo*, and showed a strong *E(top)* phenotype (not shown). The *E(top)* mutations *D20*, *D29*, *D30*, *D44*, *D50*, *D67*, *D69*, *D76*, *D82*, *D85* and *D86* were all lethal in combination with the *top*^{18A} deficiency (see Table 2, Column 9). Because of their unusual wing vein defects, it was of interest to determine if *E(top)*s *D30* and/or *D82* produced an embryonic lethal phenotype when placed over the *top*^{18A} deficiency. The genetic construct of

Table 3.

Inter se Complementation Data for the *Enhancers of torpedo* of the Second Chromosome.

Table 3 shows the results obtained from the *inter se* complementation crosses involving the *E(top)*s of the second chromosome. In all cases, the genotype of interest was *b pr cn top*^{1} *bw E(top) "X" / b pr cn top*^{1} *bw E(top) "Y"*. If flies were present, the result was recorded as viable (V); if these flies were absent, the result was recorded as lethal (L). If less than 1% viability was seen in a given cross, it was recorded as semi-lethal (S). See text for discussion.

(1) These combinations produced defects in the L3 wing vein (phenotypes not shown).

(2) These combinations produced defects in both the L3 and L5 wing veins, in addition to the to the defects of the L4 vein (phenotypes not shown).

E(top)	D86	D85	D82	D76	D69	D67	D50	D44	D30	D29	D20	D106	D56	D9	D112	D84	D81	D59	D43	D3
D3	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V
D43	V	V	V (1)	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V	
D59	V	V	V (1)	V	V	V	V	V	V	V	V	V	V	V	V	V	V	V		
D81	V	V	V	V	V	V	V	V	V	V (1)	V	V	V	V	V	V	V			
D84	V	V	V	V (1)	V	V	V	V	V	V	V	V	V (1)	V	V	V				
D112	V	V	V	V	V	V	V	V	V	V	V (1)	V	V	V	V					
D9	V	V	V	V	V	V	V	V	V	V	V	V	V	L						
D56	V	V	V (1)	V (1)	V (1)	V (1)	V	V (1)	V	V (2)	V	V	L							
D106	V	V	V (1)	V (1)	V	V	V	V	V	V	V	L								
D20	L	L	L	L	L	L	L	L	L	L	L									
D29	L	L	L	L	L	L	L	L	L	L										
D30	L	L	L	L	L	L	L	L	L											
D44	L	L	L	L	L	L	S (1)	L												
D50	L	L	L	L	L	L	L													
D67	L	L	L	L	L	L														
D69	L	L	L	L	L															
D76	L	L	L	L																
D82	L	L	L																	
D85	L	L																		
D86	L																			

interest was *b pr cn top*^{1} *bw E(top)* / + + + *top*^{18A} + +. For both *E(top)*s *D30* and *D82*, an embryonic lethal phenotype was observed, and was indistinguishable from that of a severe *faint little ball* phenotype (not shown). None of the other combinations were examined for their phenotypes over the deficiency.

Star (*S*), is a known enhancer of *torpedo* (J. Price, unpublished data; Raz and Shilo, 1993; this work), and, in a *top*^{1} mutant background, *Star* produces eye and wing vein phenotypes strikingly similar to some of the *E(top)*s of chromosome 2 (J. Price, personal communication). Flies *trans*- heterozygous for an allele of *Star* (S^{17B}) and individual *E(top)*s were constructed by crossing S^{17B} / *CyO* males to *b pr cn top*^{1} *bw E(top)* / *CyO* virgin females.

If any of the *E(top)*s of the second chromosome were allelic to *Star*, then S^{17B} / *E(top)* flies would not be seen amongst the progeny, as dominant, loss-of-function mutations in *Star* behave as recessive lethals. In all 20 cases, the S^{17B} / *E(top) trans*-heterozygotes were viable [and displayed a strong *E(top)* phenotype], an indication that no new alleles of the *Star* locus had been recovered in the screen and that the *E(top)*s represented loci distinct from the *Star*.

The 20 *E(top)*s were also tested against each other in *inter se* complementation tests. Progeny of each cross were examined for the presence or, conversely, the absence of *b pr cn top*^{1} *bw E(top)"X"* / *b pr cn top*^{1} *bw E(top)"Y"* flies. If these flies were present, the result was recorded as viable (V); if these flies were not present, the result was recorded as lethal (L). Results of all crosses are reported in Table 3.

Note that in the *top*^{18A} and *inter se* complementation tests, the various mutant combinations are not reported as having "complemented" or "failed to complement". Rather, these combinations are reported as either viable or lethal, the reasons for doing so will be presented in the **Discussion** section of this thesis.

Section II.

The *Enhancers of torpedo* of the Third Chromosome.

From the four screens described in **Materials and Methods**, a total of five dominant *Enhancers of torpedo*, or *E(top)*s, were mapped to the third chromosome. In this section, the characterization of these third-chromosomal *E(top)*s is presented.

Male flies of genotype *top*^{1} *bw / CyO ; E(top) / TM3* were mated individually to *top*^{1} *bw / CyO* virgin females. Flies of genotype *top*^{1} *bw / top*^{1} *bw ; E(top) / +* were selected from amongst the F_1 progeny. As with the *E(top)*s of the second chromosome, these *top*^{1} *bw / top*^{1} *bw ; E(top) / +* flies were subjected to the same phenotypic examinations in order to assess the ability of the *E(top)* to enhance phenotypes associated with the *top*^{1} mutation. While none of the third-chromosomal *E(top)*s showed any discernible enhancement of either the maternal effect or compound eye phenotypes of *top*^{1}, these *E(top)*s were characterized based on their ability to enhance the defects of the *top*^{1} wing, as seen by the presence of fourth longitudinal wing vein (L4) gaps. These mutations were further characterized in two complementation analyses. Genetic map positions were determined for three of these *E(top)*s.

Wing Phenotypes.

As was the case with the *E(top)*s of the second chromosome, all of the third-chromosomal *E(top)*s were recovered for their ability to produce L4 gaps in the wing of a *top*^{1} mutant fly. *E(top)*s *2-2*, *2-21* and *C1-2* all cause a partial disruption of the L4 wing vein, with a segment between the posterior cross vein (PCV) and the margin of the wing being deleted (Figure 4, C, D and E, respectively). While *E(top)*s *D105* and *K24* were initially recovered for their ability to produce L4 disruptions, these mutations have weakened sufficiently such that they no longer display this phenotype. Since *E(top) K24* is homozygous viable, it was desirable to see what effect 2 copies of this mutation would have on the *top*^{1} phenotype. Flies homozygous for both *top*^{1} and *E(top) K24* were present within the balanced stock, and while the phenotype was not entirely penetrant, these flies did, in fact, show a partial disruption of the L4 wing vein (Figure 4F). The

Figure 4.

Enhancement of *top*^{1} Wing Vein Defects by Third-Chromosomal *Enhancers of torpedo*.

(A), a wild type (*ORE R*) wing. Vein designations are marked on the wing, with L2, 3, 4 and 5 = second, third, fourth and fifth longitudinal veins; ACV and PCV are the anterior and posterior cross veins, respectively.

(B), a *top*^{1} *bw* / *top*^{1} *bw* wing, showing the deletion of the ACV.

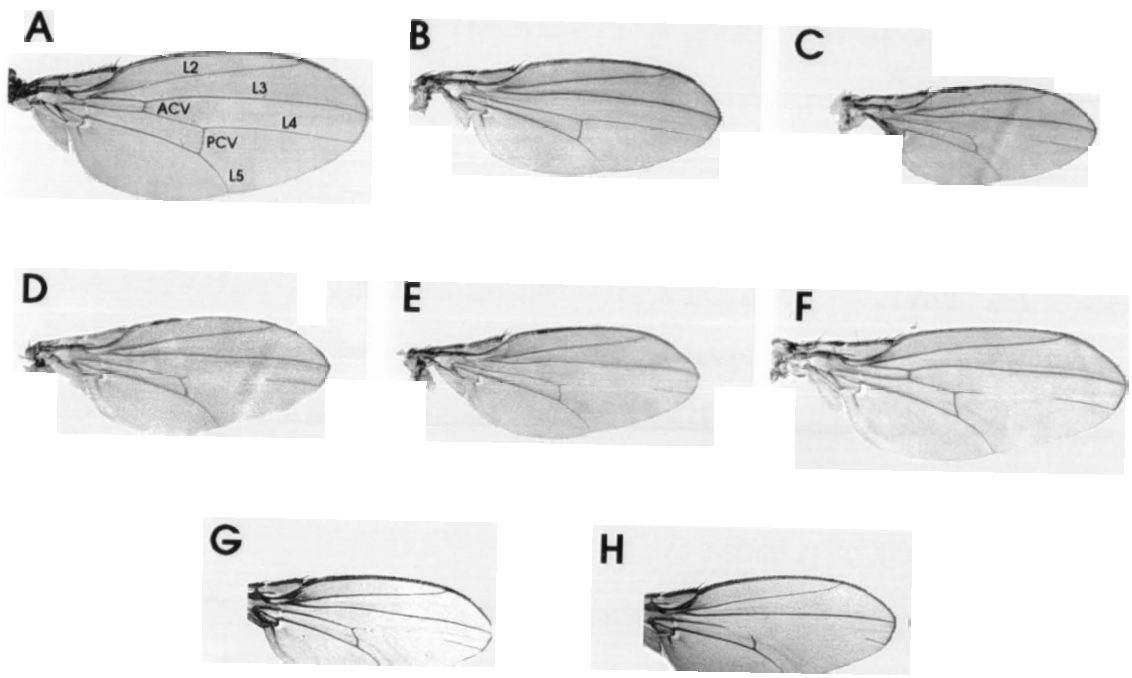
(C), a *top*^{1} *bw* / *top*^{1} *bw* ; *E(top) 2-2* / + wing. The ACV is deleted, a gap in L4 is present, and there is a moderate distortion of the PCV. The ampulla is bent under the wing in this photograph.

(D), a *top*^{1} *bw* / *top*^{1} *bw* ; *E(top) 2-21* / + wing, with an ACV deletion, and a gap in the L4 vein. There is also a moderate distortion of the PCV.

(E), a *top*^{1} *bw* / *top*^{1} *bw* ; *E(top) C1-2* / + wing, showing an ACV deletion, along with an L4 gap and a very slight distortion of the PCV.

(F), a *top*^{1} *bw* / *top*^{1} *bw* ; *Bnf* / *Bnf* wing. These wings show a moderate gap in the L4 vein. Flies heterozygous for the *Bnf* mutation no longer show an L4 defect in a *top*^{1} mutant background. The phenotype of homozygotes is not completely penetrant.

(G), a *top*^{1} / *top* ^{2C82} wing, showing deletion of the ACV, as well as an L4 gap and a slight distortion of the PCV, and (H), a *top*^{1} / *Df(2R)top* ^{3F18} wing. Again, the ACV is deleted, and there is a much stronger L4 defect, as the L4 gap is now proximal to the PCV, the upper portion of which is absent. Wings in (G) and (H) are shown to demonstrate the effects of reducing *torpedo* activity below that observed in *top*^{1} / *top*^{1} homozygotes. The wings in photographs (G) and (H) were obtained from J. Price.


A
L2
L3
ACV
L4
PCV
L5
B
C
D
E
F
G
H

E(top) K24 mutation has been renamed *Bare naked fly*, or *Bnf*. Henceforth, the *Bnf* notation will be used in reference to the *E(top) K24* mutation.

Complementation Analyses.

The five *E(top)*s of the third chromosome were first examined for viability when homozygous. Stocks were constructed which involved the removal of the top^1 allele from the original balanced stock. The *E(top) / TM3* stocks were then screened for homozygotes. Only *Bnf* was found to be homozygous viable, and flies carrying two copies of the *Bnf* mutation displayed a subtle phenotype. The postvertical (PV) bristles of the head are apparently affected by the *Bnf* mutation, in that a significant proportion of *Bnf* homozygotes (> 75%) show a reduction in the length of the PV bristles, and, in some cases, deletions of one or both of these bristles were detected (phenotypes not shown). E(top)s *2-2*, *2-21*, *C1-2* and *D105* were found to behave as recessive embryonic lethals, but no discernible cuticle defects were observed in these embryos (phenotypes not shown).

Two different complementation analyses were carried out to further characterize these mutations. The third-chromosomal *E(top)*s were tested against four *Enhancers of sevenless*, or *E(sev)*s, reported in Simon *et al.* (1991); the *E(top)*s were also tested against each other in *inter se* complementation tests.

It has been reported that the members of the sevenless signaling cassette mediate the signaling of the EGF receptor (torpedo) in *Drosophila* (Simon *et al.*, 1991; Doyle and Bishop, 1993; Diaz-Benjumea and Hafen, 1994). With this in mind, the *E(top)*s were tested against the *E(sev)*s of the third chromosome: *E(sev)3A*, *E(sev)3B*, *E(sev)3C/Ras1* and *E(sev)3D*. In all 20 cases, the *E(top) / E(sev) trans*-heterozygotes were found to be viable, with none of these flies displaying any distinguishing phenotypes (not shown).

The third-chromosomal *E(top)*s were also tested against each other in *inter se* complementation tests. All *trans*-heterozygous combinations were viable (results shown

Table 4.

Inter se Complementation Data for the *Enhancers of torpedo* of the Third Chromosome.

Table 4 shows the results obtained from the *inter se* complementation tests involving the *E(top)*s of the third chromosome. In all cases, the genotype of interest was *E(top)* "*X*" / *E(top)* "*Y*" (homozygous wild type for *torpedo*). If these flies were present, the result was recorded as viable (V); if these flies were absent, the result was recorded as lethal (L). See text for discussion.

(1) These *trans*-heterozygous combinations showed a deletion of the anterior cross vein (ACV).

(2) These *trans*-heterozygous combinations showed gaps in the L4 wing vein.

(3) No phenotype was observed in this *trans*-heterozygous combination.

(4) Flies homozygous for the *Bnf* mutation show reduction or deletion of the postvertical bristles.

E(top)	*Bnf*	*D105*	*C1-2*	*#2-21*	*#2-2*
#2-2	V (1)	V (1)	V (1)	V (1,2)	L
#2-21	V (2)	V (2)	V (2)	L	
C1-2	V (3)	V (1)	L		
D105	V (1)	L			
Bnf	V (4)				

in Table 4), with all but one of the mutant combinations showing a discernible phenotype. *E(top) 2-2*, in combination with *E(top)*s *C1-2*, *D105* and *Bnf*, showed the deletion of the anterior cross vein (ACV). The combination of *E(top)*s *2-2* and *2-21* not only produced a deletion of the ACV, but an L4 gap was also seen in these flies. The *E(top) 2-21* mutation produced L4 gaps when in combination with the *E(top) C1-2*, *D105* and *Bnf* mutations. Deletions of the ACV were also seen in *E(top) C1-2 / E(top) D105* and *E(top) D105 / Bnf* mutant combinations. None of the above phenotypes are shown. No phenotype was observed in flies *trans*-heterozygous for *E(top) C1-2* and *Bnf*.

Some *Enhancers of torpedo* are Dominant Enhancers of *Hairless*.

Specific crosses were set in the balancing of the *E(top) D105* mutation. Male flies of genotype top^1 *bw / * top^1 *bw ; E(top) D105 / +* , which showed L4 defects, were individually mated to top^1 *bw / CyO ;* H^{21C} */ TM3* virgin females. Males of genotype top^1 *bw / * top^1 *bw ; E(top) D105 /* H^{21C} were selected from amongst the F_1 progeny. These males were mated to top^1 *bw / CyO ;* H^{21C} */ TM3* virgins in order to create a balanced stock. It was noticed, however, that the top^1 *bw / * top^1 *bw ; E(top) D105 /* H^{21C} males displayed an interesting phenotype, in that these flies had far fewer macrochaetae (bristles) than seen in flies which were carrying only the H^{21C} mutation. Wing vein (L4) defects were also seen in these flies, but at the time, it was uncertain as to precisely which mutation(s) were causing the observed effects.

Wild type flies possess 40 bristles distributed over the head and thoracic regions (Figure 5B). Dominant, loss-of-function mutations in *Hairless*, or *H*, are known to cause a "bristle loss/double socket" phenotype as well as wing vein defects (Ashburner, 1982; Bang *et al.*, 1991). In flies which are heterozygous for the H^{21C} allele, the number of bristles is reduced to an average of 25 (Figure 5D). Flies heterozygous for mutations in *Hairless* also show a subtle wing vein defect, in that the fifth longitudinal wing vein (L5) is shortened, such that it fails to reach the margin of the wing (Figure 5C). In

Figure 5.

Some *Enhancers of torpedo* are Dominant Enhancers of *Hairless*.

(A), a wild type (*ORE R*) wing.

(B), showing the bristle pattern of a wild type fly. There are 40 bristles visible over the head and thorax, along with a full complement of microchaetae.

(C) shows a H^{21C} / + wing. The L5 wing vein fails to reach the wing margin.

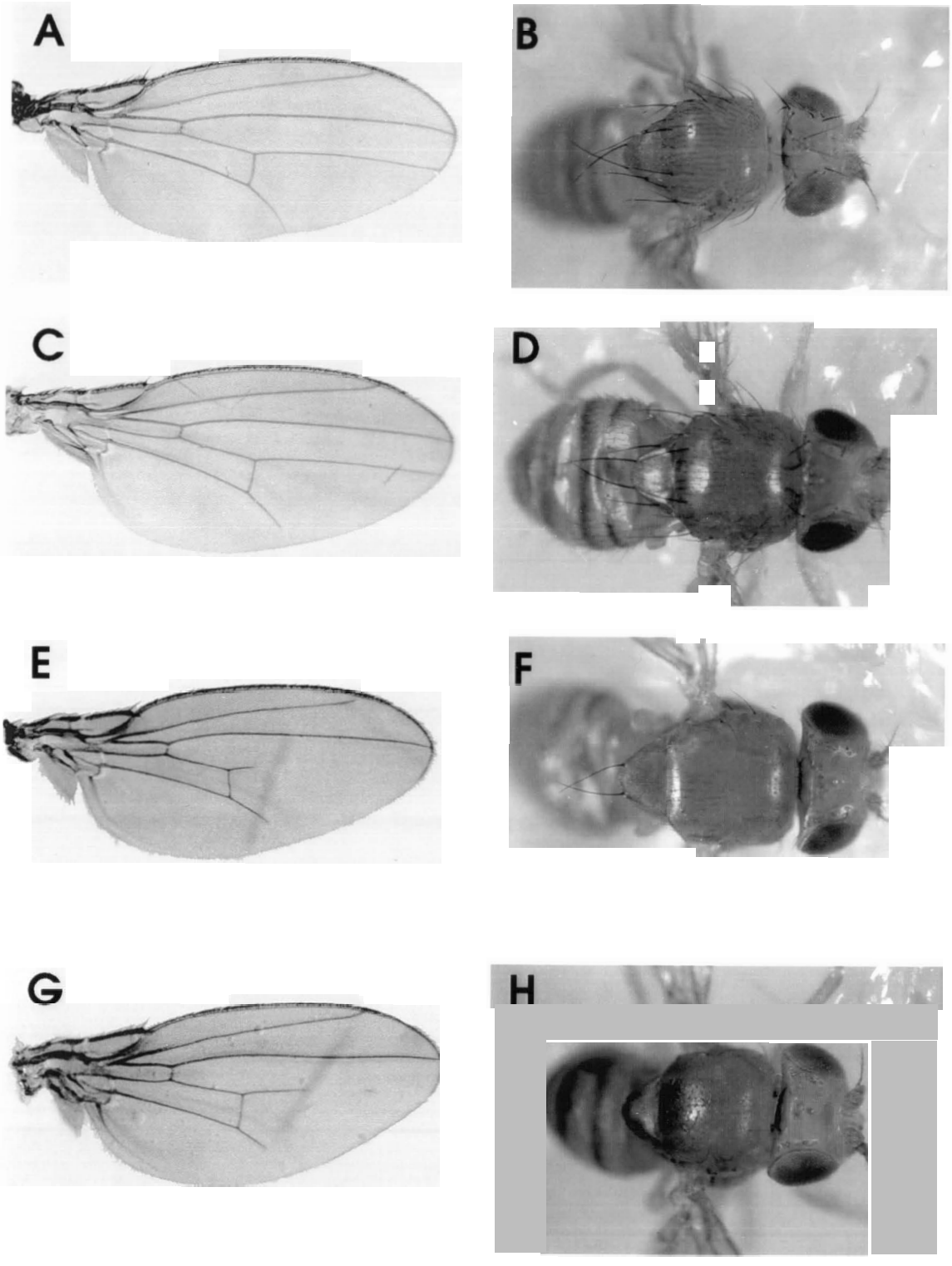
(D), the bristle pattern of a H^{21C} / + heterozygote. An average of 15 bristles are deleted from the head and thorax. A few microchaetae are missing from the thorax.

(E) shows an *E(top) 2-21* / H^{21C} wing. The L4 vein is deleted distal to the PCV, and the L5 vein is shorter than that seen in a H^{21C} / + wing.

(F), the bristle pattern of an *E(top) D105* / H^{21C} fly. Only seven bristles remain on this particular fly, and a greater number of microchaetae are missing.

(G) shows the wing of a *Bnf* / H^{21C} fly. The L4 wing vein is deleted distal to the PCV, and the L5 vein is slightly shorter than in a H^{21C} heterozygote.

(H), the bristle pattern of a *Bnf* / H^{21C} fly. Only one bristle remains intact and virtually all the microchaetae are missing, hence the *Bare naked fly* (*Bnf*) designation.


A
B
C
D
E
F
G
H

top^1 *bw* / *top*^{1} *bw* ; *E(top) D105* / H^{21C} flies, the number of bristles is reduced even further than in H^{21C} / + flies, such that only 10 bristles remain on average.

Because the *E(top) D105* mutation exhibited *Enhancer of Hairless* [*E(H)*] activity, it was of interest to determine whether the other third-chromosomal *E(top)*s displayed similar activity. When *E(top)* / *TM3* stocks were obtained for all five *E(top)* mutations, they were crossed into a H^{21C} / *TM3* stock to test for any enhancement of the *Hairless* phenotypes (again in the absence of the *top*^{1} mutation). While *E(top)*s *2-2* and *C1-2* did not show any enhancement of either the "bristle-loss" or wing vein phenotypes of H^{21C}, the *E(top)* mutations *2-21*, *D105* and *Bnf* showed differential abilities to enhance the phenotypes of the H^{21C} allele. Table 5 summarizes all *E(top)* and *E(H)* phenotypes.

The *E(top) 2-21* mutation enhanced only the wing vein phenotype of H^{21C}, producing an L4 gap which extended from the margin of the wing to a point just distal to the posterior cross vein (PCV); the L5 vein of the *E(top) 2-21* / H^{21C} *trans*-heterozygotes was also slightly shorter than that of H^{21C} / + flies (Figure 5E). No enhancement of the "bristle-loss" phenotype could be associated with the *E(top) 2-21* mutation.

Flies *trans*-heterozygous for *E(top) D105* and H^{21C} showed no enhancement of the H^{21C} wing vein defect. However, the number of bristles in *E(top) D105* / H^{21C} flies was reduced to an average of 10 (Figure 5F), compared to 25 bristles seen in a H^{21C} / + fly (Figure 5D). The "bristle-loss" phenotype of *E(top) D105* / H^{21C} flies was not affected by the absence of the *top*^{1} mutation, as *top*^{1} *bw* / *top*^{1} *bw* ; *E(top) D105* / H^{21C} flies and + / + ; *E(top) D105* / H^{21C} flies showed no difference in bristle number, indicating that the enhancement of the H^{21C} bristle defect was a result of the activity of the *E(top) D105* mutation.

The *Bnf* mutation showed enhancement of both the "bristle-loss" and wing vein phenotypes of H^{21C}. In the wing, *Bnf* produced a gap in the L4 wing vein reaching from the margin of the wing to a point distal to the PCV, as well as a slight shortening of the

Table 5.

Summary of Phenotypes for the *Enhancers of torpedo* of the Third Chromosome.

Table 5 shows a summary of the phenotypes produced by the *E(top)*s of the third chromosome. All phenotypes are for *top*^{1} *bw* / *top*^{1} *bw* ; *E(top)* / + flies, unless otherwise noted. Both males and females were scored for these phenotypes.

(1) These mutations initially showed disruptions of the L4 wing vein; these defects are no longer seen. See text for discussion.

(2) *E(top) K24/Bnf* shows the L4 defect as a homozygote, i.e.: *top*^{1} *bw* / *top*^{1} *bw* ; *Bnf* / *Bnf*.

(3) In H^{21C} / + flies, an average of 15 bristles from the head and thorax are deleted, leaving 25 bristles intact. The number of bristles remaining in either *D105* / H^{21C} or *Bnf* / H^{21C} flies is an average, reported out of the 25 bristles seen in a H^{21C} / + fly. These phenotypes were observed in *E(top)* / H^{21C} flies.

(4) The L4 defects were not seen in H^{21C} / + flies; the L5 wing vein of *D105* / H^{21C} and *Bnf* / H^{21C} flies is shorter than that seen in H^{21C} / + flies.

(5) Phenotype observed in *E(top)* / *E(top)* homozygous flies (homozygous wild type for *torpedo*).

(6) These mutations behave as embryonic lethals, but no obvious cuticle defects are observed in these embryos.

(7) One or both of the postvertical bristles are either reduced in overall length or deleted entirely (phenotypes not shown).

	Phenotype of	Displays E(H)	Number of	Phenotype of	Genetic map	Phenotype when
E(top)	E(top) wings	activity (3)	bristles left (3)	E(H) wings (3)	position	homozygous (5)
#2-2	L4 gap	no	N/A	N/A	N/D	embryonic lethal (6)
#2-21	L4 gap	yes (wings only)	N/A	L4, L5 short (4)	3-15.9	embryonic lethal (6)
C1-2	L4 gap	no	N/A	N/A	N/D	embryonic lethal (6)
D105	wild type (1)	yes (bristles only)	10 out of 25 (3)	N/A	3-87.1	embryonic lethal (6)
K24(Bnf)	L4 gap (2)	yes (wings, bristles)	2 out of 25 (3)	L4, L5 short (4)	3-64.2	bristle defects (7)

L5 vein, relative to a H^{21C} / + wing (Figure 5G). The *Bnf* mutation also displays very strong *E(H)* activity with respect to its enhancement of the "bristle-loss" phenotype of H^{21C}. In *Bnf* / H^{21C} *trans*-heterozygotes, only one or two bristles remain on average (Figure 5H), relative to the 25 bristles seen in H^{21C} / + flies. While *Bnf* removes almost all of the macrochaetae from the fly's head and thorax, this mutation also deletes nearly all the microchaetae from the thoracic region. Therefore, a *Bnf* / H^{21C} fly is virtually devoid of bristles, and for this reason, the name *Bare naked fly*, or *Bnf*, has been assigned to this locus.

Mutations in *Hairless* are Dominant *Enhancers of torpedo*.

Apart from the five *E(top)*s described above, two other mutations were recovered which also displayed *E(top)* activity, *E(top)*s *D57* and *D73*. In addition, these two mutations showed a reduced number of head and thoracic bristles, a phenotype strikingly similar to that of loss-of-function mutations in the *Hairless* gene. The *E(top) D57* and the *E(top) D73* mutations were therefore crossed into a H^{21C} / *TM3* stock to test whether or not they would complement the H^{21C} allele. No *E(top) D57* / H^{21C} or *E(top) D73* / H^{21C} flies were found amongst the F_1 progeny, it was concluded that *E(top)*s *D57* and *D73* were, in fact, new alleles of *Hairless*, given that strong hypomorphic alleles of *Hairless* act as recessive lethals (Bang *et al.*, 1991). These mutations have been renamed H^{D57} and H^{D73}, respectively.

With respect to their abilities in enhancing the top^1 phenotype, both top^1 *bw* / top^1 *bw* ; H^{D57} / + and top^1 *bw* / top^1 *bw* ; H^{D73} / + flies produced gaps in the L4 wing vein which reached from the PCV to a point close to the margin of the wing; the PCV was also slightly distorted in these wings (Figure 6, C and D). The H^{21C} allele was also isolated as an *E(top)* mutation, and leads to the presence of L4 gaps in the wings of top^1 *bw* / top^1 *bw* ; H^{21C} / + flies (J. Price, unpublished results; this work, Figure 6B).

Figure 6.

Mutations in *Hairless* and *Star* Enhance top^1 Wing Vein Defects.

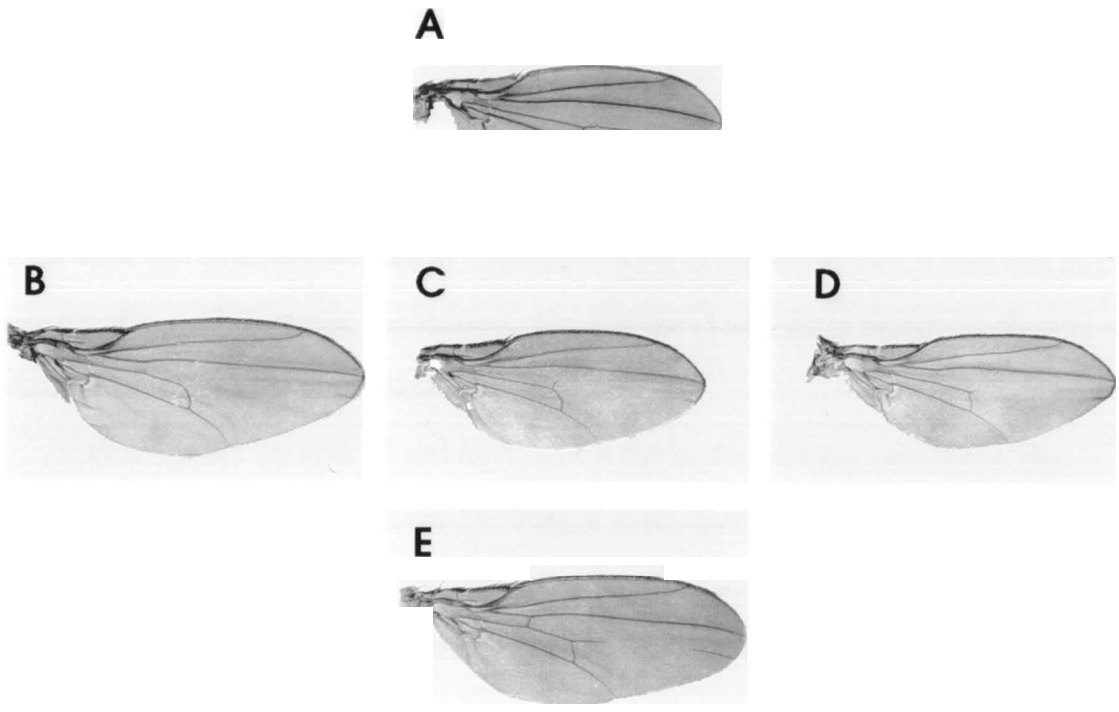
(A) shows a top^1 *bw* / top^1 *bw* wing. The ACV is deleted.

(B), a top^1 *bw* / top^1 *bw* ; H^{21C} / + wing showing the deletion of the ACV and a gap in the L*A* vein distal to the PCV, which is slightly distorted.

(C), showing a top^1 *bw* / top^1 *bw* ; H^{D57} / + wing with ACV, L*A* and PCV defects very similar to those seen in photograph (B).

(D) , a top^1 *bw* / top^1 *bw* ; H^{D73} / + wing. Again, this allele of *Hairless* behaves almost identically to the *Hairless* mutations pictured in (B) and (C).

(E) shows a S^1 top^1 *bw* / + top^1 *bw* wing. There is a gap in the L*A* vein.


A
B
C
D
E

Effects of Triple-Mutant Combinations on *top*^{1} Wing Vein Defects.

All studies of the enhancement of *torpedo* phenotypes has looked at the effects double-mutant combinations, so it was of interest to determine what effects triple-mutant combinations might have on the *top*^{1} phenotype, and to see if more severe phenotypes could be produced. Flies of genotype *S*^{1} *top*^{1} *bw* / + *top*^{1} *bw* ; *E(top)* / + and *top*^{1} *bw* / *top*^{1} *bw* ; *E(top)* / *H*^{21C} were constructed using all five of the third-chromosomal *E(top)* mutations.

Before crossing the *E(top)*s into the *Star/torpedo* background, it was necessary to determine if any of these mutations showed interaction(s) with *Star*. Flies doubly heterozygous for *S*^{2N23} and *E(top) 2-21* showed disruptions of the L2, L4 and L5 wing veins, such that these veins failed to reach the margin of the wing; the L4 vein was most strongly affected (phenotype not shown). None of the other *E(top)* mutations showed any interactions with this allele of *Star*.

Flies mutant for both *S*^{1} and *top*^{1} show a gap in the L4 wing vein which lies between the PCV and the margin of the wing; a slight distortion of the PCV is also seen (Figures 6E, 7B). All five *E(top)*s were able to increase the severity of both the L4 defect and the disruption of the PCV. The *E(top)* mutations *2-2*, *2-21*, *C1-2* and *Bnf* all showed moderate increases in the size of the L4 gap, while *E(top) D105* produced the greatest increase in the size of the L4 gap, extending it to a point proximal to the PCV (Figure 7, D, F, H, J, and L). Apart from enhancing the severity of the L4 defect, *E(top) 2-2* and *E(top) 2-21* also produced gaps in the third longitudinal (L3) wing vein (Figure 7, D and F, respectively). Gaps in the L3 wing vein are not seen in flies mutant only for *Star* and *torpedo*, meaning that the appearance of such a defect was the result of interactions between the *Star*, *torpedo* and *E(top)* mutations.

Flies of genotype *top*^{1} *bw* / *top*^{1} *bw* ; *H*^{21C} / + also have a gap in the L4 vein which reaches from the PCV to a point close to the margin of the wing; a partial

Figure 7.

Effects of Triple-Mutant Combinations on *top*^{1} Wing Vein Defetcs.

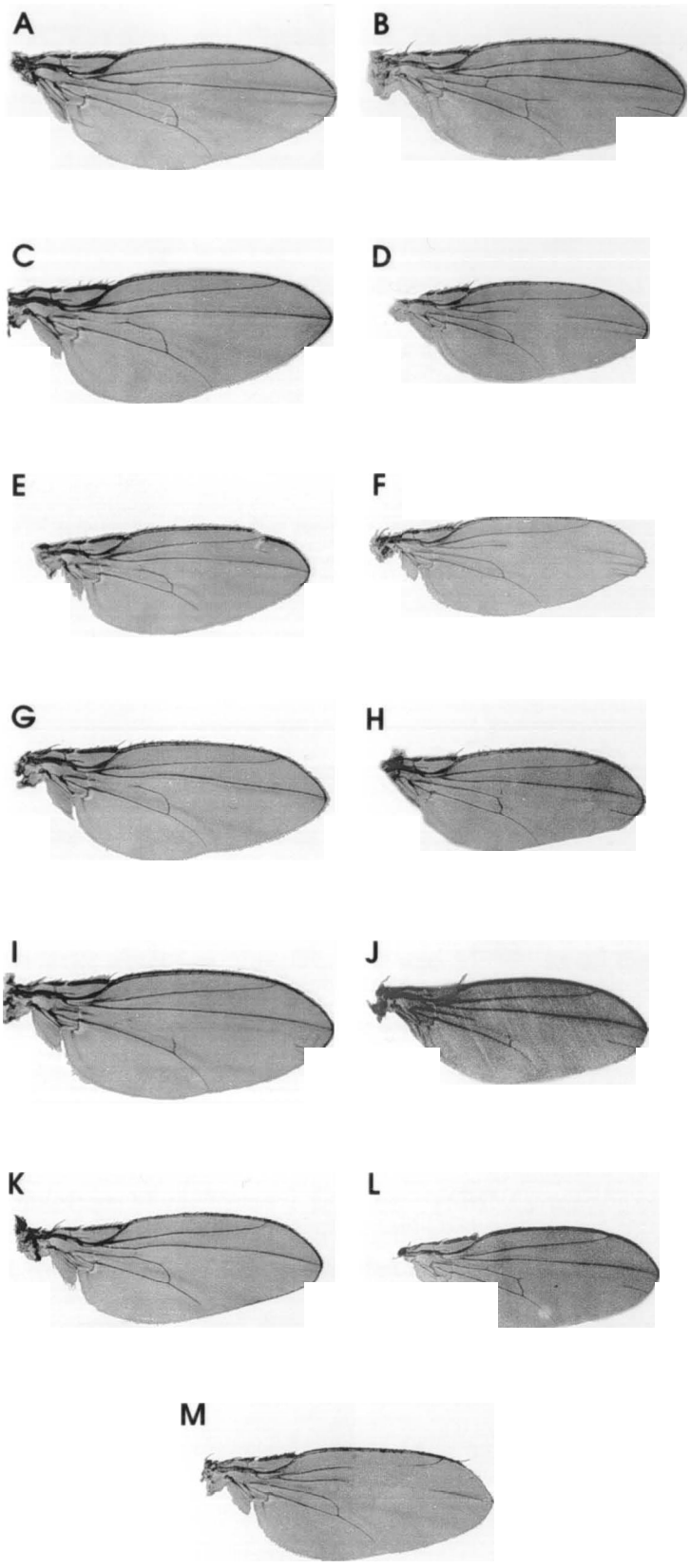
(A), a wing mutant for *top*^{1} and *H*^{21C}. This wing shall be referred to as the *torpedo/Hairless* background; the genotype is *top*^{1} *bw* / *top*^{1} *bw* ; *H*^{21C} / +.

(B), a wing mutant for *S*^{1} and *top*^{1}. This wing shall be referred to as the *Star/torpedo* background; the genotype is *S*^{1} *top*^{1} *bw* / + *top*^{1} *bw*.

For photographs (C), (E), (G), (I) and (K), the genotype is the *torpedo/Hairless* background plus *E(top) 2-2* (C), *E(top) 2-21* (E), *E(top) C1-2* (G), *E(top) D105* (I) and *E(top) K24/Bnf* (K). *E(top)*s *2-2*, *2-21* and *C1-2* all produce moderate increases in the severity of the L*4* defect, deleting an additional segment proximal to the PCV. The L*4* defects are strongly enhanced with the *E(top) D105* mutation; the upper portion of the PCV is also deleted. The *Bnf* mutation produced a modest increase in the size of the L*4* defect.

For photographs (D), (F), (H), (J) and (L), the genotype is the *Star/torpedo* background plus *E(top) 2-2* (D), *E(top) 2-21* (F), *E(top) C1-2* (H), *E(top) D105* (J) and *E(top) K24/Bnf* (L). *E(top)*s *2-2* and *2-21* show an increase in the severity of the L*4* defect as well as producing a distortion of the PCV and gaps in the L3 wing vein. The *E(top) D105* mutation proved to impart a strong enhancement of the L*4* defect, as well as a deletion of the upper portion of the PCV. The *Bnf* mutation produced a moderate increase in the size of the L*4* gap as well as slight distortion of the PCV.

(M) shows a wing mutant for *Star*, *torpedo* and *Hairless*; the genotype of this wing is *S*^{1} *top*^{1} *bw* / + *top*^{1} *bw* ; *H*^{21C} /+. The L*4* defect is somewhat more severe than either *Star* or *Hairless* can produce on its own. Gaps in the L2 and L3 wing veins are also observed.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disruption of the PCV is also seen (Figure 7A). As was the case with the *Star/torpedo* mutant background, the addition of any of the five *E(top)* mutations was able to increase the severity of the L4 defect in the *torpedo/Hairless* mutant background. The *Bnf* mutation produced only a slight increase in the size of the L4 gap, deleting the small portion close to the wing's margin such that the entire length of the L4 vein distal to the PCV was now deleted (Figure 7K). The *E(top) 2-2*, *2-21* and *C1-2* mutations were able to increase the size of the L4 gap such that it extended just proximal to the PCV (Figure 7, C, E and G, respectively); *E(top) 2-2* also increased the degree of disruption of the PCV. *E(top) D105* proved to have the strongest effect on the L4 wing vein, in that it was able to delete nearly the entire wing vein (Figure 7I). The *E(top) D105* mutation also increased the severity of the PCV disruption, such that only the lower half of the PCV was left intact.

To examine what effect a combination of both the *Star* and *Hairless* mutations would have on the top^1 phenotype, it was first necessary to establish if *Star* and *Hairless* interacted with each other in the absence of mutant *torpedo* activity. The H^{21C} mutation was tested against three alleles of *Star*, S^1, S^{17B} and S^{2N23}. In all three double-heterozygotes, the *Star* mutation was found to enhance the wing vein defects of H^{21C}, causing a further reduction in the length of the L5 wing vein and producing a moderate shortening of the L4 wing vein (phenotypes not shown). To introduce mutant *torpedo* activity, flies of genotype S^1 top^1 *bw* / + top^1 *bw* ; H^{21C} / + were constructed. A gap in the L4 wing vein was to be expected, seeing that both mutations independently cause L4 defects in a top^1 background. The L4 defect was not much stronger than either of the S^1 or H^{21C} mutations would have produced on its own in combination with the homozygous top^1 mutation; the L4 vein was deleted from the PCV to the margin of the wing, with no increase in the strength of the PCV disruption. Defects in two other wing veins were also detected, as seen in the partial disruption of both the second (L2) and third (L3)

longitudinal wing veins (Figure 7M). The disruption of the L2 and L3 veins is not seen in connection with either *Star* or *Hairless* in their respective enhancement of the top^1 phenotype, a possible indication that *Star* and *Hairless* are capable of acting in a synergistic manner to enhance the wing vein defects associated with the top^1 mutation.

Genetic Mapping of *E(top) 2-21*, *E(top) D105* and *Bare naked fly*.

Because the *E(top)* activity of both the *E(top) D105* and *Bnf* mutations had weakened to such an extent that they no longer showed an L4 defect, it was only possible to map them for their *Enhancer of Hairless*, or *E(H)*, activity. *E(top) 2-21* was also mapped as an *E(H)* mutation, simply because the mapping protocol for the *E(H)*s produced the desired results one generation sooner than mapping the same mutation as an *E(top)* mutation, All three mutations were mapped according to the procedure outlined in the **Material and Methods** section of this thesis.

Twenty-four recombinant males were tested for *E(H)* activity in the mapping of the *E(top) 2-21* mutation. The *E(H)* phenotype of *E(top) 2-21* was mapped to a position of 15.9 map units on the left arm of the third chromosome, based on 10 recombination events between the *roughoid* (3-0.0) and *hairy* (3-26.5) phenotypic markers.

The *E(top) D105* and *Bnf* mutations both mapped to the right arm of the third chromosome. Using 23 recombinant males, the *E(H)* activity of *E(top) D105* was mapped to a position of 87.1 map units based on 11 crossover events between the phenotypic markers *ebony* (3-70.7) and *claret* (3-100.7). For the mapping of *Bnf*, 70 recombinant males were tested for their ability to enhance *Hairless*. The *E(H)* activity of *Bnf* was localized to a position of 64.2 map units, based on four recombination events between the markers *stripe* (3-62.0) and *ebony*.

It is necessary to correlate the *E(top)* activities of these mutations with their *E(H)* activities. An attempt was made with the *Bnf* mutation, but the phenotype is not completely penetrant (even in flies homozygous for *Bnf*), so no reliable connection could

be made between the two activities of the *Bnf* mutation. The *E(top) 2-21* and *E(top) D105* mutations were not tested for the correlation of their *E(top)* and *E(H)* activities. *E(top) D105* no longer shows its *E(top)* phenotype. Too few recombinants of *E(top) 2-21* were obtained; therefore, a statistically significant correlation between its *E(top)* and *E(H)* activities could not be established.

The question of whether or not the apparent lethality of *E(top) 2-21* and *E(top) D105* could be removed from these mutations' *E(H)* activity by recombination was also addressed. All those recombinant chromosomes which displayed the *E(H)* activity of either the *E(top) 2-21* or *E(top) D105* mutation were lethal, implying the lethality of the recombinant chromosome could be correlated with the *E(H)* activity of these mutations.

While it is possible to map the *2-2* and *C1-2* mutations as *E(top)*s, too few recombinant chromosomes were recovered to provide a statistically reliable map position. It should also be possible to map *E(top)*s *2-2*, *2-21*, *C1-2* and *D105* as recessive lethals.

Discussion.

From the four screens designed to generate dominant *Enhancers of torpedo*, a total of 25 such mutations were recovered. Twenty of these mutations were mapped to the second chromosome; five were mapped to the third chromosome.

The *Enhancers of torpedo* of the Second Chromosome.

The 20 *E(top)*s of the second chromosome were subjected to extensive genetic and phenotypic analysis, with the objective of determining the number of loci that are represented by these 20 mutations.

The Possibility of Compound Mutations in *torpedo*.

A new class of *torpedo* mutation may have been recovered in the screens, the compound allele. The screens were carried out in a top^1 mutant background; males homozygous for the *b pr cn* top^1 *bw* chromosome were mutagenized. It is, therefore, quite possible that additional mutation may have been superimposed onto the top^1 allele, which may serve to further reduce the activity of *torpedo*. There also exists the distinct possibility that a given mutagenized chromosome has sustained two mutational events. One mutation could have been induced in either an *E(top)* locus or in top^1 (resulting in the creation of a compound *torpedo* allele), and the other in an essential locus (resulting in a recessive lethal allele).

The *Enhancers of torpedo* May Be Allelic to *torpedo*.

There is some question as to how many genes are represented by the 20 *E(top)*s of the second chromosome. Evidence obtained from the phenotypic and genetic analysis of these mutations suggests that the entire collection of 20 *E(top)*s represent new, compound alleles of *torpedo*.

The frequency at which *torpedo* alleles can be recovered can be estimated from Clifford and Schüpbach (1989). In their experiments, three screens were conducted, all using 50 mM EMS and involving a total of 8739 chromosomes. Thirteen alleles of *torpedo* were recovered in these screens, for an observed frequency of one *torpedo* allele

for every 672 chromosomes. Four screens were conducted in our lab, comprising a total of just over 25,000 chromosomes. Since our screens also employed 50 mM EMS, it is possible to predict the number of compound *torpedo* mutations that would be expected in our screens. From the frequency of *torpedo* mutations recovered in Clifford and Schüpbach (1989), our screens would be expected to yield approximately 37 alleles of *torpedo*. In this situation, these mutations would represent compound alleles, as the top^1 mutation was already present. Since only 20 *E(top)* mutations were recovered on the second chromosome, it is apparent that these 20 mutations fall short of the expected number of 37 new *torpedo* mutations. It is therefore quite likely that at least some, or perhaps all of the 20 *E(top)*s of the second chromosome are new alleles of *torpedo*, with a secondary lesion superimposed onto the top^1 allele. If secondary mutations have been induced in *torpedo*, then all 20 putative *E(top)*s would automatically represent compound *torpedo* alleles.

Phenotypic Evidence.

With a few exceptions (as detailed below), the entire set of phenotypes displayed by the 20 *E(top)*s of the second chromosome - summarized in Table 2 - can be associated with mutations in *torpedo*. The increased roughening of the eye, the production of gaps in the L4 wing vein, the varying degrees of ventralization of the eggshell and the low fecundity of mutant females are all found associated with mutations in *torpedo* (Clifford and Schüpbach, 1989; Price *et al.*, 1989). However, it must be acknowledged that compound alleles of *torpedo* could also be capable of producing exceptional phenotypes.

The embryonic lethal phenotypes of *E(top)*s *D20*, *D29*, *D30*, *D44*, *D50*, *D67*, *D69*, *D76*, *D82*, *D85* and *D86* (Figure 2) are similar to the phenotypes of a weak or moderate *faint little ball* (*flb*) mutant, the embryonic lethal phenotype of *torpedo*. While both classes of embryos display varying degrees of disruption of the head cuticle, there is a slight difference between them. The embryos of the 11 mutations have no ventral

denticle bands or dorsal hairs, traces of which can be seen in some *flb* embryos (Clifford and Schüpbach, 1989, 1992; Raz and Shilo, 1993). It is possible that a compound *torpedo* mutation may have the ability to affect different aspects of *torpedo* function as it relates to embryonic development. Two constituent mutations within a *torpedo* allele could differentially interfere with processes that are required for the formation of the ventral denticles and dorsal hairs, giving rise to embryos with naked epidermis.

In the top^{18A} complementation test, these 11 *E(top)*s were all lethal in *trans* to the top^{18A} deficiency (see below for further discussion); the terminal phenotypes of two of these mutations were examined. With both the *E(top) D30* and *E(top) D82* mutations, the genetic construct *b pr cn* top^{1} *bw E(top)* / + + + top^{18A} + + showed a phenotype which was indistinguishable from that of a severe *flb* embryo, an indication that at least *E(top)*s *D30* and *D82* could be allelic to *torpedo*. However, the possibility that these two *E(top)* mutations could represent a locus distinct from *torpedo* must also be considered.

Genetic Evidence.

Two kinds of complementation analyses were used in the genetic characterization of these 20 mutations. The results of both the top^{18A} and *inter se* complementation tests are consistent with the possibility that the 20 *E(top)*s are new mutations of *torpedo* superimposed onto the top^{1} allele during mutagenesis (see above).

The 20 *E(top)*s were first tested against a deficiency of *torpedo*, *Df(2R)*top^{18A} which removes only the *torpedo* locus (Price *et al.*, 1989). The *trans*-heterozygous combination of top^{1} / *Df(2R)*top^{18A} is viable, but the activity of *torpedo* is reduced to such a degree that strong phenotypic defects are visible; these phenotypes are essentially the same as those of the nine *E(top)*s which were viable over the top^{18A} deficiency (phenotypes not shown). Eleven *E(top)*s were lethal in *trans* to the top^{18A} deficiency. This pattern of viability and lethality is typical of *torpedo* mutations. Weak or moderate *torpedo* alleles are easily viable when placed over a deficiency, in that there is still

enough *torpedo* activity present to allow the genetic construct to survive to adulthood. Strong *torpedo* alleles may reduce the activity of *torpedo* to such a degree that when they are placed in *trans* to the deficiency, their combination is lethal. If such a combination is strong enough, it may be capable of producing the embryonic phenotypes described above, as seen with *E(top)*s *D30* and *D82*.

Inter se complementation tests were also used to assist in the genetic characterization of these mutations. Again, the *inter se* complementation data presented in Table 3 is consistent with the notion that the entire collection of *E(top)*s could be alleles of *torpedo*. Viability or lethality of each *trans*-heterozygous combination would depend on the strength of the *torpedo* alleles in question. Combinations of weak and/or moderate alleles of *torpedo* could easily survive if the two alleles, together, possessed enough *torpedo* activity to permit viability. Alternatively, the two presumptive alleles of *torpedo* could be strong enough to reduce *torpedo* activity below its minimum threshold level, resulting in lethality.

There are several minor discrepancies within the *inter se* complementation data. *E(top)*s *D44* and *D50* are both homozygous lethal mutations, yet their *trans*-heterozygous combination is weakly viable (see legend for Table 3). While it is possible that different second-site mutations have been induced in essential/*E(top)* loci distinct from *torpedo* on both the *E(top) D44* and *E(top) D50* chromosomes, it is possible that these two *E(top)* mutations are compound alleles of *torpedo*. It has been reported that known lethal alleles of *torpedo* are capable of complementing each other when heterozygous (Clifford and Schüpbach, 1989; Raz *et al.*, 1991), so it is quite possible that *E(top)*s *D44* and *D50* are allelic to the *torpedo* locus.

Three other mutations, *E(top)*s *D9*, *D56* and *D106*, are all homozygous lethal and all are viable over the top^{18A} deficiency, observations which may indicate that these three *E(top)*s are mutations in loci distinct from *torpedo*. However, these three mutations may

very well be weak or moderate compound alleles of *torpedo*, each associated with its own second-site lethal mutation, accounting for their *inter se* viability and their viability over the top^{18A} deficiency.

The above genetic and phenotypic evidence, are consistent with the possibility that all 20 *E(top)*s of the second chromosome may be new, compound alleles of *torpedo*.

Some *Enhancers of torpedo* May Represent Novel Loci.

While there is considerable evidence in support of the idea that the 20 *E(top)* mutations of the second chromosome are compound mutations of *torpedo*, there are observations which suggest that at least some of these *E(top)*s may represent mutations in loci distinct from *torpedo*.

Wing Phenotypes.

All 20 *E(top)*s were recovered for their ability to produce gaps in the L4 wing vein of a top^1 mutant fly. Since they were isolated, *E(top)*s *D3*, *D9*, *D84* and *D112* have weakened sufficiently that they no longer display the L4 defect. It is possible that these mutations are not completely penetrant and/or that they may have accumulated modifier mutations on their respective chromosomes. While the L4 defect is typical of heteroallelic combinations of *torpedo* mutations, there are three mutations which display additional wing vein defects which have not been seen with previous *E(top)* phenotypes (Clifford and Schüpbach, 1989; Price *et al.*, 1989; J. Price, personal communication). There are three second-chromosomal *E(top)* mutations which are known to produce novel wing vein defects, *E(top)*s *D20*, *D30* and *D82*. In addition to L4 defects, these mutations produce gaps in the L3 wing vein, and *E(top) D82* also showed gaps in the L2 vein. Although these defects have never been observed in flies either homozygous or *trans*-heterozygous for alleles of *torpedo*, it is possible that compound alleles of *torpedo* could produce these phenotypes, if, for instance, the compound mutations were particularly disruptive to the activity of *torpedo* as it pertains to wing development.

While L2 and L3 defects have not been seen in association with previous *torpedo* alleles, they are, however, associated with a mutation in a separate locus, *rolled*, which is known to interact with *torpedo*. The *rolled* gene product (MAP kinase) has been shown to function downstream of the torpedo receptor, and loss-of-function mutations in *rolled* are known to produce a severe *E(top)* phenotype in the wings of flies mutant for hypomorphic alleles of *torpedo* (Brunner *et al.*, 1994; Diaz-Benjumea and Hafen, 1994). In these wings, the L2 vein shows a small gap, while the L3, L4 and L5 veins are entirely absent. In addition, clonal analysis has shown that several members of the sevenless signaling cassette - *Drk*, *Sos*, *Ras1* and *raf* (as well as *rolled*) - are required for torpedo receptor function during epidermal development as it pertains to wing vein pattern formation (Diaz-Benjumea and Hafen, 1994). Thus it is apparent that both *torpedo* and members of the torpedo pathway are required for proper wing vein pattern formation, even though this information is difficult to obtain without the benefit of clonal analysis. Therefore, the wing vein defects produced by *E(top)*s *D20*, *D30* and *D82* are consistent with those produced by mutations in other genes that participate in the torpedo pathway.

Complementation Analyses.

While the results obtained from the top^{18A} and *inter se* complementation tests can be readily interpreted from the point of view that the 20 *E(top)*s are compound alleles of *torpedo*, the possibility exists that these 20 mutations represent novel *E(top)* loci.

In the complementation tests employing the top^{18A} deficiency, the viability of the *b pr cn* top^{1} *bw E(top)* / + + + top^{18A} + + genotype depends on the strength of the *E(top)* mutation. Weaker *E(top)* mutations may not be strong enough to reduce the activity of *torpedo* to a sub-viable level, but instead may produce an *E(top)* phenotype in the viable adult. Stronger *E(top)* mutations could reduce *torpedo* activity sufficiently to generate synthetic lethality. Synthetic lethality would be produced by the genetic combination of the *E(top)* with a sufficiently strong *torpedo* mutant background. A

combination of this nature could also produce the *flb*-like embryonic lethal phenotype, as was the case with both *E(top)*s *D30* and *D82* (see above), implying that these two *E(top)* mutations may be loci distinct from *torpedo*. Such synthetic lethality is observed in flies heterozygous for mutations in *Star* and *trans*-heterozygous for top^{1} and top^{18A} (J. Price, personal communication).

The results of the *inter se* complementation tests can also be interpreted as being consistent with the idea that these mutations represent novel *E(top)* mutations. Again, the viability of the *trans*-heterozygous combinations is dictated by the strength of the *E(top)*s involved. Viability would be expected if both *E(top)*s were relatively weak mutations. Such combinations may not be sufficient to reduce the activity of *torpedo* to a level below that required for viability, and produce *E(top)* phenotypes in the adult flies. If any of the *trans*-heterozygous combinations was lethal, then it would appear that the two *E(top)*s are strong enough to reduce the activity of *torpedo* to such a level that it would be incompatible with viability.

A number of the *trans*-heterozygous combinations in the *inter se* complementation tests showed unusual wing vein defects not seen with known *E(top)* phenotypes (see Table 3 for a complete listing). The presence of such novel wing vein defects would indicate that at least some of the *E(top)* mutations involved may represent mutations in loci distinct from *torpedo*.

Embryonic Lethal Phenotypes.

As stated previously, *E(top)*s *D20*, *D29*, *D30*, *D44*, *D50*, *D67*, *D69*, *D76*, *D82*, *D85* and *D86* produce lethality during embryogenesis (when homozygous), showing phenotypes similar to those of weak to moderate *flb* embryos. While compound *torpedo* mutations may be responsible for such phenotypes, distinct *E(top)* mutations could also account for these phenotypes.

Both *flb* embryos and embryos homozygous for mutations in *Star* show reductions

in the amount of ventral denticle material. When embryos double mutant for *Star* and a weak *flb* allele are constructed, a synergistic interaction is observed, such that the embryo assumes a severe *flb* phenotype (Raz and Shilo, 1993) with virtually no ventral cuticle. Thus, a mutation in a locus separate from *torpedo* is capable of affecting *torpedo* function in the embryo resulting in the enhancement of the *torpedo* embryonic phenotype. Given this fact, it is possible that the 11 embryonic lethal *E(top)*s may be interacting with *torpedo* in a synergistic manner, affecting *torpedo* function in such a way that the genotypic construct results in embryonic lethality and produces a phenotype where the ventral denticles and dorsal hairs are absent.

There is one piece of evidence which strongly suggests that at least one locus distinct from *torpedo* has been identified. The *E(top) D76* mutation has been mapped by K. Fitzpatrick of our lab; she has determined that *E(top) D76* maps distal to the *speck* locus (2-107.0), placing *E(top) D76* in close proximity to the extreme right tip of the second chromosome. Further efforts are currently underway to more precisely define the genetic map position of *E(top) D76*. The phenotypes imparted by the *E(top) D76* mutation are virtually identical to ten other mutations, *E(top)*s *D20*, *D29*, *D30*, *D44*, *D50*, *D67*, *D69*, *D82*, *D85* and *D86* (see Table 2). If these phenotypic similarities are of any significance, then it is possible that a total of 11 alleles of one locus have been identified. However, the other ten mutations would have to be mapped before any such statements can be made conclusively.

Even though the *E(top)*s of the second chromosome have been subjected to rigorous genetic and phenotypic analysis, the exact number of loci represented by these 20 mutations remains unknown. Preliminary evidence suggests that at least one locus distinct from *torpedo* has been recovered in the screens. With the benefit of additional genetic mapping data, the number of genes represented in the collection of second-chromosomal *E(top)*s may be established.

The *Enhancers of torpedo* of the Third Chromosome

Greater attention has been given to the five *E(top)*s of the third chromosome. The phenotypes produced by these mutations are regarded as significant because they cannot be new alleles of *torpedo*.

Wing Phenotypes.

All five *E(top)*s on the third chromosome were recovered for their ability to produce L4 defects in a top^1 mutant wing (Figure 4). Heteroallelic combinations of top^1 and stronger alleles of *torpedo* are capable of producing L4 gaps of various sizes (Figure 4, G and H). Any mutation which is capable of producing gaps in the L4 vein of a top^1 wing would be called an *E(top)* mutation. The five mutations of the third chromosome are, by virtue of their chromosomal position, genes distinct from *torpedo*, and therefore represent definite *E(top)* mutations.

The *E(top) D105* mutation has weakened to such a degree that the L4 defects are no longer apparent, due most likely to very low penetrance or to the accumulation of modifier mutations on the *E(top) D105* chromosome. The *Bare naked fly* (*Bnf*) mutation - previously called *E(top) K24* (see below) - behaved in a similar fashion, showing L4 defects when it was first recovered, but with time, these defects were not seen anymore, probably for the same reasons as with *E(top) D105*. Because the *Bnf* mutation was found to be homozygous viable, it was of interest to see if two copies of the *Bnf* mutation were able to produce L4 defects. Flies of genotype top^1 *bw* / top^1 *bw* ; *Bnf* / *Bnf* were obtained from within the stock which did, in fact, display gaps in the L4 wing vein (Figure 4F). However, not all flies showed these L4 defects, suggesting that the phenotype of the *Bnf* mutation is not completely penetrant. It would appear, then, that the *Bnf* mutation is indeed an *E(top)* mutation, but a relatively weak one, as two copies of the mutation are required to produce the *E(top)* phenotype.

Complementation Analyses.

Because members of the sevenless signaling cassette are known to interact with the *torpedo* locus (Simon *et al.*, 1991; Doyle and Bishop, 1993; Diaz-Benjumea and Hafen, 1994), it was necessary to determine if mutations in any of these known third-chromosomal loci had been recovered in the screens. The mutations *E(sev)3A*, *E(sev)3B*, *E(sev)3C/Ras1* and *E(sev)3D* reported by Simon *et al.* (1991) were used in this experiment. All *trans*-heterozygous combinations were viable, and none of the five *E(top)*s showed any discernible phenotype when *trans*-heterozygous with any of the four *E(sev)* mutations. These results would indicate that the *E(top)*s were probably not allelic to any of the *E(sev)*s used in this test.

The *inter se* complementation tests proved somewhat more revealing. The *E(top)* mutations *2-2*, *2-21*, *C1-2* and *D105* are all homozygous lethal; only *Bnf* is viable in the homozygous condition. All *trans*-heterozygous combinations were viable, suggesting that these five *E(top)*s each represent a separate mutation; in other words, mutations in five different genes had been recovered on the third chromosome. While it is possible that some of these mutations may be allelic to each other, and while the lethality of these *E(top)*s may be due to a second-site mutation elsewhere on the third chromosome, their phenotypic patterns (as described below) indicate that the collection of *E(top)*s on the third chromosome represents five separate mutations.

The combinations of the five *E(top)*s in the *inter se* crosses produced some interesting wing vein defects. A variety of wing vein defects, such as L4 gaps, ACV deletions and PCV distortions, were noticed in all *trans*-heterozygous combinations, except for that of *E(top) C1-2 / Bnf* (see **Results** and Table 5 for a complete detailing of all phenotypes). It was interesting to see these defects, as these crosses were performed in the absence of the top^1 mutant allele. Thus these five mutations are all capable of causing defects in the wing vein pattern, even in the absence of mutant *torpedo* activity.

The Genetic Mapping of *E(top) 2-21*, *E(top) D105* and *Bare naked fly*.

Genetic map positions were determined for *E(top)*s *2-21*, *D105* and *Bnf*. The mutation *E(top) D105* was mapped to a position of 3-87.1 map units, and the *Bnf* mutation was mapped to position of 3-64.2 map units. The significance of these two map positions will be discussed below.

The *E(top) 2-21* mutation was mapped to a genetic location of 3-15.9 map units, which places the *E(top) 2-21* mutation very close to the *Ras2* gene (3-[15]). Experiments were initiated to establish if *E(top) 2-21* was an allele of the *Ras2* locus. The *E(top) 2-21* mutation was crossed into a strain carrying a *Ras2* deficiency. The genetic constitution of the deficiency strain is *Df(3L)GN50 / TM8 [l(3)4-DTS th st Sb e]*, and spans the cytological interval from 63F4-7 to 64C13-15. This deficiency shall be referred to as *Df(3L)Ras2* for the purposes of this discussion.

The *2-21 / Df(3L)Ras2 trans*-heterozygotes were found to be viable, a surprising observation, given that *E(top) 2-21* is a recessive lethal. This observation would indicate that the *E(top) 2-21* mutation is outside the region uncovered by the deficiency and therefore, not an allele of *Ras2*. However, a low percentage (<15%) of flies displayed L4 gaps not seen in *Df(3L)Ras 2 / +* flies, suggesting that *E(top) 2-21* and the *Ras2* deficiency may be (weakly) interacting in a dominant fashion in *trans*-heterozygotes. The *Ras2* deficiency also displayed weak *E(H)* activity when placed in *trans* to the H^{21C} allele (data not shown). Because *Ras1* mutants have been reported to act as weak suppressors of the *Ellipse* rough eye phenotype (Simon *et al.*, 1991), both the *Ras2* deficiency and the *E(top) 2-21* mutation were tested for the ability to suppress the *Ellipse* phenotype, on the assumption that *Ras1* and *Ras2* mutants might behave in a similar manner. Flies which were heterozygous for both Elp^{1} and *E(top) 2-21* or *Df(3L)Ras2* were found to show a weak suppression of the *Ellipse* phenotype (data not shown). Both the $Ras1^{e2F}$ mutation and the *Ras2* deficiency were tested for their ability to enhance the

top^1 phenotype; no interactions were observed (data not shown). Given that the complementation tests show that *E(top) 2-21* is probably not an allele of the *Ras2* locus, the phenotypes displayed by *E(top) 2-21* suggest that a mutation in a previously unidentified locus may have been recovered, and that this locus may possess some *Ras*-like properties.

Some *Enhancers of torpedo* are Dominant Enhancers of *Hairless*.

An allele of the *Hairless* gene, H^{21C}, was used to balance the *E(top) D105* mutation. Flies heterozygous for the H^{21C} mutation show a reduction in the number of bristles from 40 in the wild type to an average of 25 in H^{21C} / + flies (Figure 5D); in addition, the L5 wing vein of these flies fails to reach the margin of the wing (Figure 5C). It was noticed that *top*^{1} *bw* / *top*^{1} *bw* ; *E(top) D105* / H^{21C} flies had more pronounced wing vein defects and showed a significant reduction in the number of bristles, as compared to a H^{21C} / + fly. Since these phenotypes could not be attributed to the *E(top) D105* mutation, it was obvious that at least two of the above mutations were interacting in some way. A stock of genotype *E(top) D105* / *TM3* was constructed and subsequently tested for *Enhancer-of-Hairless* , or *E(H)*, activity. Only the bristle defects were seen in *E(top) D105* / H^{21C} flies, indicating two things: first, that the reduction in bristle number was due strictly to the interaction of *E(top) D105* and H^{21C} (Figure 5F), and second, that the wing vein defects in the triple mutant were the result of a synergistic interaction between *top*^{1}, *E(top) D105* and H^{21C} (Figure 7I).

The other four *E(top)*s were also tested for their ability to enhance *Hairless*, but only *E(top)*s *2-21* and *K24* displayed any *E(H)* activity. While the *E(top) 2-21* mutation was found to enhance only the wing vein defects of H^{21C} (Figure 5E), *E(top) K24* was capable of enhancing both the wing vein and bristle defects of H^{21C} (Figure 5, G and H). Because of the severe reduction in bristle number imparted by the *E(top) K24* mutation, this locus has been renamed *Bare naked fly*, or *Bnf*. For the purposes of this discussion,

the *Bnf* notation will be used in reference to the *E(top) K24* mutation.

With respect to the enhancement of *Hairless* wing vein defects, The *E(H)* activity of the *E(top) 2-21* and *Bnf* mutations could simply be produced by a direct reduction in the activity of Hairless. Hypomorphic mutations in *Hairless* are known to produce defects in the L4 and L5 wing veins such that they fail to reach the margin of the wing (Bang *et al.*, 1991). The allele of *Hairless* used in this work, H^{21C}, affects only the L5 wing vein (Figure 5C). It is possible that the *E(top) 2-21* or *Bnf* mutations may be acting to decrease the activity of *Hairless*, but as it is unknown whether *E(top) 2-21* or *Bnf* are loss- or gain-of-function mutations, their mechanisms of action are uncertain.

E(top) 2-21 and *Bnf* may be involved in a pathway required to stimulate the activity of Hairless, which is a putative transcriptional regulator (Bang and Posakony, 1992; Maier *et al.*, 1992; see below). In this respect, the gene products of *E(top) 2-21* and *Bnf* would be acting as positive activators of *Hairless*. If the activity of an upstream component were reduced, i.e., a loss-of-function mutation in either *E(top) 2-21* or *Bnf*, it is conceivable that *Hairless* would suffer a corresponding reduction in activity, and the enhancement of *Hairless* wing vein defects would result. Alternatively, the activities of *E(top) 2-21*$^+$ and *Bnf*$^+$ may be to restrict the expression of *Hairless*; in this scenario, *E(top) 2-21*$^+$ and *Bnf*$^+$ would act as negative regulators of *Hairless*. A gain-of-function mutation in either *E(top) 2-21* or *Bnf* would over-restrict the expression of *Hairless*, and the activity of *Hairless* would be reduced to an even greater extent. The combination of the hypermorphic mutations in *E(top) 2-21* or *Bnf* with a hypomorphic *Hairless* allele would reduce the activity of the Hairless protein to such a degree that an *E(H)* phenotype is produced. Since it is not currently possible to distinguish between these two potential mechanisms of action, the exact role of either the *E(top) 2-21* or *Bnf* mutations and their interaction with *Hairless* in the wing remains unknown.

There exists another mutation in *Drosophila* which is known to modify the

activity of *Hairless*; this locus is called *Suppressor of Hairless*, or *Su(H)*. The *Su(H)* locus is known to be involved in two stages of the determination of the sensory organ precursor (SOP) cell fate in the developing peripheral nervous system (PNS). *Su(H)* is a neurogenic locus, as null mutations in *Su(H)* cause too many cells to adopt the SOP cell fate. Loss-of-function mutations in this locus behave as suppressors of hypomorphic *Hairless* alleles, while duplications of *Su(H)*$^+$ and gain-of-function *Su(H)* mutations behave as dominant enhancers of loss-of-function *Hairless* mutations (Ashburner, 1982; Furukawa *et al.*, 1991, 1992; Schweisguth and Posakony, 1992, 1994).

The SOP cell gives rise to a fixed lineage of four cells in the developing PNS, a neuron and three accessory cells. Two of these accessory cells are sister cells, the trichogen and the tormogen, which give rise to the shaft of the bristle and the socket, respectively. The activity of *Su(H)*$^+$ is required to limit the expression of the SOP cell fate, implying that *Su(H)*$^+$ is playing some inhibitory role in the development of the SOP cell. As well, *Su(H)*$^+$ is required for the determination of the socket-producing tormogen cell in the later stages of SOP development. Hypomorphic *Su(H)* mutations produce a "double-shaft" phenotype. The reduction of *Su(H)* activity is unable to limit the expression of the SOP cell fate, and, in later steps, the trichogen cell fate is favoured, resulting in the appearance of doubled bristles. The reduction in *Su(H)* activity may result in the transformation of a tormogen cell into a second trichogen cell (Schweisguth and Posakony, 1994). Gain-of-function mutations in *Su(H)* over-restrict the expression of the SOP cell fate, resulting in a reduced number of SOP cells, and in later stages the conversion of the trichogen into a second tormogen cell; the net effect is a "bristle-loss" and "double-socket" phenotype (Schweisguth and Posakony, 1994).

Hairless is also involved in the determination of SOP cell fate. Mutations in the *Hairless* gene confer phenotypes upon the SOP cell and its lineage that are the inverse of those seen with mutations in the *Su(H)* locus. Gain-of-function mutations in *Hairless*

produce a neurogenic phenotype, as too many SOP fates are specified, and later, the trichogen cell fate is overexpressed, resulting in the "double-shaft" phenotype. Loss-of-function mutations of *Hairless* produce both the "bristle-loss" and "double-socket" phenotypes. The "bristle loss" phenotype results from the failure to specify and/or execute the SOP cell fate, and the doubling of the socket is a result of the transformation of the trichogen into a second tormogen cell (Bang *et al.*, 1991; Bang and Posakony, 1992). The phenotypes produced by hypermorphic mutations in *Hairless* are the same as those seen in association with hypomorphic mutations in *Su(H)*, and the phenotypes seen with hypomorphic mutations in *Hairless* are strikingly similar to the phenotypes of hypermorphic mutations in *Su(H)*. From these observations, it has been proposed that *Su(H)* may be a negative regulator of *Hairless* activity (Ashburner, 1982; Schweisguth and Posakony, 1992), and it has since been shown that *Su(H)* and *Hairless* are acting antagonistically to control alternative cell fates in the developing PNS (Schweisguth and Posakony, 1994).

The *Su(H)* locus has been cloned and was found to encode the homologue of the mouse recombination-signal binding protein, J_K-RBP, whose product was thought to bind to the recombination signal sequence of immunoglobulin J_K segments (Furukawa *et al.*, 1991, 1992; Schweisguth and Posakony, 1992). It has since been shown that the mouse Su(H) protein does not bind to the J_K gene recombination signal sequences (Tun *et al.*, 1994; Henkel *et al.*, 1994). Instead, J_K-RBP has recently been shown to be identical to the *C promoter-binding factor 1* (*CBF1*) gene which encodes a DNA-binding protein that mediates the transactivation of viral and cellular genes with the Epstein-Barr virus nuclear antigen, EBNA2 (Dou *et al.*, 1994; Henkel *et al.*, 1994). The Su(H) protein of *Drosophila* is now regarded as the homologue of CBF1 (Fortini and Artavanis-Tsakonas, 1994). The Su(H) protein also shares a motif with known integrases and recombinases, a motif that is directly involved with the catalysis of recombination. The presence of a

recombinase motif in the Su(H) protein has lead to the suggestion that Su(H) may specify SOP cell fate through some kind of recombination event, but this has not yet been demonstrated (Furukawa *et al.*, 1991,1992; Schweisguth and Posakony, 1992). Recent studies have established that the sequence similarity of the Su(H) protein to the integrase/recombinase domain has no functional significance *in vivo* (Schweisguth *et al.*, 1994). *Hairless*, too, has been cloned and was found to encode a novel basic protein which shows some homology to a family of proteins known to be involved in transcriptional regulation (Bang and Posakony, 1992; Maier *et al.*, 1992). Thus, it seems that both *Hairless* and *Su(H)* are involved in the specification of the SOP cell fate by participating in some form of DNA binding and/or transcriptional control (Schweisguth and Posakony, 1992; Tun *et al.*, 1994).

The ability of gain-of-function mutations in *Su(H)* to behave as dominant enhancers of the *Hairless* "bristle-loss" phenotype could be explained as follows. The gain-of-function *Su(H)* mutation would over-restrict the expression of the SOP cell fate, and favour the tormogen cell fate in later stages. Hypomorphic alleles of *Hairless* would also fail to specify the SOP cell, as well as favouring the tormogen cell fate in later stages. Placed together, these two mutations would result in a dramatic reduction in the number of cells adopting the SOP cell fate, and favour the tormogen cell fate in later stages, which would greatly reduce the number of bristles, producing a severe "bristle-loss", or *Enhanced-Hairless* [*E(H)*] phenotype.

The *E(H)* phenotype described above is very similar to the severe "bristle-loss" phenotypes of both the *E(top) D105* and *Bnf* mutations (Figure 5, F and H). While the exact nature of the *E(H)* phenotype produced by these two mutations is currently unknown, it is possible to suggest several modes of action for both the *E(top) D105* and *Bnf* mutations. The potential role of the *E(top) D105* mutation shall be addressed first.

The *E(H)* activity of *E(top) D105* was mapped to a genetic position of 87.1 map

units, placing it very close to a locus called *Enhancer of split* , or *E(spl)*, which maps to a genetic position of 89.1 map units. The *E(spl)* gene is a neurogenic locus known to be involved in the determination of the SOP cell fate. Recessive, loss-of-function mutations of *E(spl)* produce a neurogenic phenotype in that too many cells adopt the SOP cell fate, while the only known dominant gain-of-function mutation, $E(spl)^{D}$, results in too few cells assuming the SOP cell fate, with more cells assuming an epidermal fate (Knust *et al.*, 1987; Preiss *et al.*, 1988; Schrons *et al.*, 1992; Tata and Hartley, 1993). From these phenotypes, the *E(spl)* locus is behaving in a very similar manner to *Suppressor of Hairless*, in that both seem to be working against the SOP cell fate (in the normal condition). Genetic interactions between *E(spl)* and *Hairless* have shown that *E(spl)* appears to be the target of the repressing activity of *Hairless*, as *Hairless*, normally specifying the SOP cell fate, would have to counteract the epidermal-specifying activity of the *E(spl)* locus (Vässin *et al.*, 1985; de la Concha *et al.*, 1988). The *E(spl)* locus has been cloned and was shown to encode no less than seven basic, helix-loop-helix(bHLH)/DNA-binding proteins, which negatively interfere with the transcriptional activation mediated by the proneural genes (Delidakis *et al.*, 1991; Knust *et al.*, 1992; Tietze *et al.*, 1992; Oellers *et al.*, 1994).

It could be suggested that if the dominant gain-of-function $E(spl)^{D}$ mutation were to be combined with a loss-of-function mutation in *Hairless*, there would be a dramatic reduction in the number of SOP cells, as the $E(spl)^{D}$ mutation would over-restrict the SOP cell fate, and loss of *Hairless* activity would fail to execute the SOP cell fate (as well as favouring the tormogen cell fate in later stages). A severe "bristle-loss" phenotype would be expected, and is the exact phenotype seen in *E(top) D105* / H^{21C} flies (Figure 5F). While it has yet to be shown conclusively, the map position of *E(top) D105* and its interactions with *Hairless* suggest that the *E(top) D105* mutation is behaving like a dominant, gain-of- function mutation in one of the bHLH proteins

encoded by the *E(spl)* locus. It must be acknowledged that while *E(top) D105* may be allelic to the *E(spl)* locus, it may also be that *E(top) D105* is a mutation in a previously unidentified locus. A simple test would be to determine if the *E(top) D105* mutation displays *E(spl)* activity. The results obtained in this work support the conclusion that the *E(top) D105* locus is somehow involved in the pathway which is required for the proper specification of the SOP cell fate in the developing peripheral nervous system, possibly through interactions with *Su(H)* and/or *Hairless*.

The genetic basis of the interaction between *Hairless* and the *Bnf* mutation is not yet clear, as it is not known whether *Bnf* is a gain- or loss-of-function mutation. However, it is possible to suggest several modes of action for the *Bnf* mutation.

The *Bnf* gene may interact with *Hairless* in two different ways. *Bnf*$^+$ may act as a negative regulator of *Hairless*$^+$. If *Bnf*$^+$ were to limit the expression of *Hairless*$^+$, a hyperactive *Bnf* gene product would serve to over-restrict *Hairless*$^+$ expression. Upon introduction of a loss-of-function *Hairless* mutation, the activity of the Hairless protein would be reduced to such a degree that an *E(H)* phenotype results. Alternatively, *Bnf*$^+$ could behave as a positive activator of *Hairless*$^+$, required as a component in a pathway needed to stimulate the activity of *Hairless*$^+$. A loss-of-function mutation in *Bnf* would result in a corresponding decrease in the activity of *Hairless*, and if the Hairless protein were already affected by a loss-of-function mutation, the overall activity of *Hairless* could be reduced to such a level that an *E(H)* phenotype is produced.

Since *Hairless* and *Suppressor of Hairless* are known to act antagonistically, the Bnf mutation could just as well be affecting the activity of the Su(H) protein. *Bnf*$^+$ and *Su(H)*$^+$ could work together to restrict the expression of the SOP cell fate, such that *Bnf*$^+$ might serve to stimulate the activity of *Su(H)*$^+$. A gain-of-function mutation in *Bnf* would result in hyperactivation of the Su(H)$^+$ protein, and produce a relative excess of *Su(H)* activity, which would then over-restrict the expression of the SOP cell fate. In

combination with a loss-of-function mutation in *Hairless*, an *E(H)* phenotype would result. Alternatively, *Bnf*$^+$ may serve to negatively regulate the activity of the Su(H)$^+$ protein. *Bnf*$^+$ could possibly encode a molecule which counteracts the inhibitory action of the Su(H)$^+$ protein, and together with the proneural activity of *Hairless*$^+$, may work to ensure that the correct number of SOP cells is established. If the *Bnf* locus were to suffer a loss-of-function mutation, then the activity of the Su(H)$^+$ protein would go unchecked, and reduce the number of cells adopting the SOP fate. If a hypomorphic *Hairless* mutation were introduced into this genetic environment, an *E(H)* phenotype would result. It is not possible to distinguish between any of these modes of action at this time.

The *Bnf* mutation displays an independent phenotype, as seen by the defects of the postvertical (PV) bristles in *Bnf* homozygotes; one or both of the PV bristles may be shortened or deleted entirely (phenotypes not shown). The loss of the PV bristles is most frequently seen in hypomorphic *Hairless* and hypermorphic *Su(H)* mutations (Bang *et al.*, 1991; Schweisguth and Posakony, 1992), thus the homozygous *Bnf* phenotype is consistent with its interactions with *Hairless* (or *Suppressor of Hairless*).

While the roles of *E(top) D105* and *Bnf* are yet to be established. It is, however, clear that *E(top) D105* and *Bnf* are somehow involved in the development of the adult peripheral nervous system. Further discussion is offered below.

Mutations in *Hairless* and *Star* Also Enhance *top*^{1} Wing Vein Defects.

Two other third-chromosomal mutations - *E(top)*s *D57* and *D73* - showing *E(top)* activity were recovered in the screens. They also showed a "bristle-loss/double-socket" phenotype similar to that of a hypomorphic allele of *Hairless*. Complementation tests later showed the *E(top) D57* and and *E(top) D73* mutations to be alleles of the *Hairless* locus. These mutations are renamed H^{D57} and H^{D73}, respectively. *Hairless* mutations enhance the *torpedo* phenotype in the wing, as seen by the ability of the H^{21C} mutation to produce L4 gaps in the wings of a *top*^{1} mutant fly; the H^{21C} allele was isolated as an

E(top) mutation in an earlier screen (J. Price, unpublished data; this work, Figure 6B). The H^{D57} and H^{D73} mutations behave almost identically to the H^{21C} allele with respect to their *E(top)* phenotypes (Figure 6, C and D, respectively). The top^1 mutation had no effect on the bristle defects of any of the *Hairless* alleles tested, nor did *Hairless* exert any influence on the eye defects or maternal effect embryonic lethal phenotype of top^1 (data not shown), so it seems that the interaction between the *torpedo* and *Hairless* loci is limited to the wing.

Very little can be said about the interaction between *Hairless* and *torpedo* based on the above phenotypes. However, it is appealing to think that a signal from *torpedo* (the EGF receptor homologue) is possibly being transmitted to the nucleus where it modifies the activity of the Hairless transcriptional regulator. Hairless would, therefore, be an excellent candidate as a potential downstream effector of *torpedo* activity.

Mutations in *Star* are also known to affect the phenotype of top^1, as seen by the presence of L4 defects in a S^1 top^1 *bw* / + top^1 *bw* fly (J. Price, unpublished data; this work, Figure 6E). Mutations in *Star* have also been shown to enhance the phenotypes of *torpedo* mutations in eye development (Kolodkin *et al.*, 1994) as well as during embryogenesis (Raz and Shilo, 1993) and oogenesis (J. Price, unpublished data). The *Star* locus has been cloned and it was found to encode a novel protein with a putative transmembrane domain (Higson *et al.*, 1993; Kolodkin *et al.*, 1994), and it is possible that there may be some interaction between the torpedo/EGF receptor protein and the *Star* gene product in the membrane. In speculation, dominant, loss-of-function mutations in *Star* may serve to decrease the signaling efficiency of the EGF receptor. When the activity of the EGF receptor is compromised by a hypomorphic mutation such as top^1, the mutant Star product may decrease the signaling efficiency of the mutant receptor even further, producing the *E(top)*/wing vein phenotype. It must be acknowledged, however, that *Star* may affect *torpedo* in some other way. Further comment will be made below.

Effects of Triple-Mutant Combinations on *top*^{1} Wing Vein Defects.

All studies in this work have so far looked at the effects of a single, second-site mutation on the *top*^{1} phenotype, but it was of some interest to determine what the effects of triple-mutant combinations might be. Experiments were initiated to see whether triple-mutant combinations can produce additional, more severe phenotypes.

Because both *Hairless* and *Star* mutations showed *E(top)* phenotypes, it was first decided to see what - if any - relationships could be drawn between *Hairless* and *Star*. Mutations in *Star* have been shown to enhance the wing vein defects of the H^{21C} allele (E.S., phenotypes not shown). However, in a *top*^{1} mutant background, *Star* and *Hairless* produce a severe *E(top)* phenotype (Figure 7M). The L4 defects were expected and observed in the wing, but L2 and L3 defects were also seen, a novel class of *E(top)* phenotype. Thus, it appears that both *Star* and *Hairless* enhance defects associated with the *top*^{1} mutation, possibly in a synergistic manner.

Two additional series of experiments were conducted, designed to examine the effects of the third-chromosomal *E(top)*s in both *torpedo/Hairless* and *Star/torpedo* double-mutant backgrounds. In both double-mutant backgrounds, each of the five *E(top)* mutations was able to increase the severity of the wing vein defects (Figure 7). With these observations, it is possible to comment on the relationship between *torpedo*, *Star*, *Hairless* and the five *E(top)*s of the third chromosome.

It has been shown that mutations in *Hairless* and *Star* are - both individually and together - capable of enhancing *top*^{1} wing vein defects (Figure 7, A, B and M). The current model of EGF receptor-mediated signaling involves the dimerization of the EGF receptor molecule (Raz *et al.*, 1991; Clifford and Schüpbach, 1994). The Star transmembrane protein, which is known to interact genetically with the torpedo receptor (Raz and Shilo, 1993; Kolodkin *et al.*, 1994; J. Price, unpublished results; this work), may be acting in the membrane at the same level as torpedo, and may serve to stabilize

the dimerized EGF receptor complex. The Hairless transcriptional regulator - residing in the nucleus - would represent a molecule very near to the end of the pathway, or Hairless may itself be the end target of this pathway.

The *inter se* complementation tests between the third-chromosomal *E(top)*s suggest that all five *E(top)* mutations may be involved in wing vein pattern formation. These same five *E(top)*s also were also able to enhance the wing vein defects in both *Star/torpedo* and *torpedo/Hairless* mutant backgrounds (Figure 7). With these observations in mind, these *E(top)*s may represent five intermediate steps in this theoretical pathway (with their precise order in the pathway being unknown). In this pathway, the *torpedo* and *Star* gene products act cooperatively in the membrane to receive, maintain, and transmit a would-be signal. The four *E(top)* mutations, *2-2*, *2-21*, *C1-2* and *K24(Bnf)* would then participate to transmit this signal to the nucleus (with the acknowledgement that these four genes may not be acting alone). As *E(top) D105* may be an allele of the *E(spl)* locus (see above), this hypothetical signal would then reach the nucleus and modify the activity of the Hairless transcriptional regulator as well as its downstream effector, the E(spl)[E(top) D105?] bHLH/DNA-binding protein (Vässin *et al.*, 1985; de la Concha *et al.*, 1988), and could possibly effect the expression of other genes involved in wing vein pattern formation.

The *Notch* locus encodes a transmembrane receptor with a large extracellular domain consisting of 36 tandem EGF-like repeats (Kidd *et al.*, 1986), and mediates the pathway which is required for the proper specification of the SOP cell fate. Other genes involved in this pathway include *Delta*, *mastermind*, *deltex*, *Hairless*, *Suppressor of Hairless* and the *Enhancer of split* complex (Furukawa *et al.*, 1992, Schweisguth and Posakony, 1992; Fortini and Artavanis-Tsakonas, 1994; Jennings *et al.*, 1994).

There is a great deal of circumstantial evidence which would implicate several of the *E(top)*s as components of a Notch-mediated pathway. The *E(H)* phenotypes of the

E(top) 2-21, *E(top) D105* and *Bnf* mutations already suggest that they could be involved in some aspect of Notch-mediated signaling (see above).

The genetic map position of *Bnf* (3-64.2) places it very close to the *Delta* locus, which resides at a genetic position of 3-66.2. The Delta protein is known to be a ligand for the Notch receptor (Fehon *et al.*, 1990; Rebay *et al.*, 1991). It has been suggested that *Bnf* could perhaps be an antimorphic allele of the *Delta* locus (J. Posakony, personal communication), but no indication of allelism between *Bnf* and *Delta* was observed in complementation tests (data not shown). Thus, the *Bare naked fly* locus may represent a previously unidentified locus.

Members of the torpedo- and Notch-mediated pathways have previously been reported to show genetic interactions. *Ellipse*, the dominant, gain-of-function allele of the EGF receptor, has been shown to behave as a strong enhancer of the *split* mutation, a recessive allele of the *Notch* locus which produces a rough eye phenotype. Baker and Rubin (1992) suggested that the *Ellipse* mutation might enhance the *split* phenotype through modifying the activity of a HLH/DNA-binding protein. Mutations in the *E(spl)* locus are known to enhance the *split* phenotype (hence the name *Enhancer of split*). As a putative allele of the *E(spl)* complex, the *E(top) D105* gene product could very well be the bHLH/DNA-binding protein whose activity is modified by *Ellipse* to produce the enhancement of the *split* phenotype, thereby establishing a possible link between the *Notch* and *torpedo* pathways. *Ellipse* also enhances the wing vein defects of both *Notch* and *Delta* mutants (Baker and Rubin, 1992). While no direct molecular interactions have been shown, the Delta and Notch proteins have been proposed as possible ligands for the EGF receptor, given that both *Delta* and *Notch* encode EGF-related proteins (Kidd *et al.*, 1986; Vässin *et al.*, 1987; Schejter and Shilo, 1989).

There is one last piece of evidence which could make further connection between the torpedo and Notch signaling pathways. *Serrate*, which encodes a transmembrane

receptor with EGF-like repeats, has been shown to act as a ligand for the Notch receptor (Rebay *et al.*, 1991; Thomas *et al.*, 1991; de Celis *et al.*, 1993). The five *E(top)* mutations are maintained over the *TM3* balancer chromosome which has an allele of *Serrate* as a phenotypic marker. There is preliminary evidence which suggests that *E(top)*s *2-21*, *C1-2* and *D105* are capable of acting as weak enhancers of the wing phenotype of *Serrate* (data not shown). Even if these results do not implicate any of these *E(top)*s in the Notch pathway, they might suggest that Serrate may be a possible ligand for torpedo. Figure 8 summarizes both the relationship between *Star*, *torpedo*, *Hairless* and the five *E(top)*s of the third chromosome, as well as the possible connection between the torpedo- and Notch-mediated pathways.

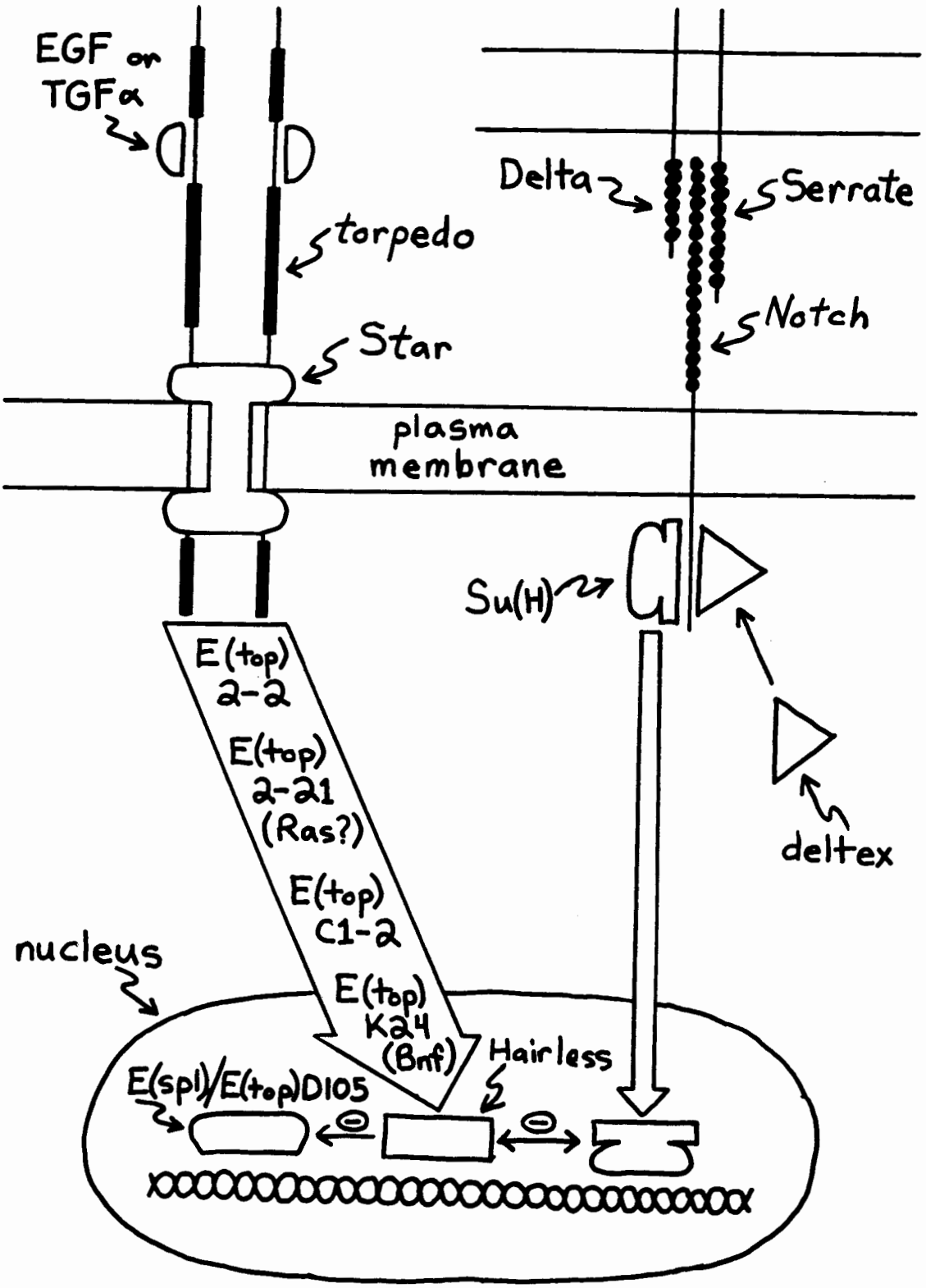
Figure 8.

Possible Relationship Between torpedo- and Notch-mediated Pathways.

In the membrane, torpedo and Star interact in some manner to transmit a signal received from the ligand, EGF (or TGFα). Downstream of the torpedo receptor are the four Enhancers of torpedo; their exact order in this hypothetical pathway is currently unknown and it is acknowledged that other factors may be participating in this pathway. This signal is ultimately transmitted to the nucleus, where it modulates the activity of the Hairless transcriptional regulator. E(top) D105 may represent a bHLH/DNA-binding protein, as *E(top) D105* is a putative mutation in the *Enhancer of split* gene complex. Genetically, *Enhancer of split* is a target of *Hairless* activity.

With the binding of Delta to its receptor, Notch, the deltex cytoplasmic protein moves to the plasma membrane where it interacts with the cytoplasmic domain of Notch, and the Suppressor of Hairless protein migrates to the nucleus, where it behaves as an antagonist of *Hairless* activity. See Fortini and Artavanis-Tsakonas (1994) for a more detailed discussion of Notch-mediated signaling. Serrate is also a ligand for Notch, but its role in Notch-mediated signaling is not as well understood as that of Delta. The *mastermind* gene product is a nuclear factor, but its precise molecular role in the Notch signaling pathway has not been established (and is not shown in this diagram).

Since both the torpedo- and Notch-mediated pathways are involved in wing vein pattern formation, and since Hairless and (perhaps) Enhancer of split are downstream effectors common to both pathways, there is evidence to suggest further relatedness between the two pathways. See text for further discussion.


EGF or
TGFα
torpedo
Star
plasma
membrane
Delta
Serrate
Notch
Su(H)
deltex
E(top)
2-2
E(top)
2-21
(Ras?)
E(top)
C1-2
E(top)
K24
(Bnf)
nucleus
Hairless
E(spl)/E(top)D105

The Screens.

This thesis presents the results of genetic screens designed to identify dominant *E(top)* mutations which were recovered for their ability to produce gaps in the L4 wing vein of a top^1 mutant fly. The presence of the L4 defect is seen with strong *torpedo* mutations and known enhancers of *torpedo*, such as *Star* and *Hairless*. This method of recovering *E(top)* mutations has been successful, given the 25 *E(top)*s characterized in this thesis.

However, purposefully selecting for L4 defects creates a problem. *E(top)* mutations will definitely be recovered, but only those which produce L4 defects. These *E(top)*s represent a class of mutations which specifically affects the functions of *torpedo* pertaining to the patterning of imaginal disc derivatives. Mutations in genes that specifically affect other aspects of *torpedo* function are likely to be missed in such a screen. For instance, mutations that interact with *torpedo* specifically during oogenesis would not be recovered, as is evident in the fact that none of the 25 *E(top)*s were able to enhance the maternal effect embryonic lethal phenotype of top^1. In order to identify mutations in genes that interact with *torpedo* during oogenesis, it would be necessary to test every mutagenized chromosome for its ability to enhance the maternal effect phenotypes of top^1. While this method would be exhaustive, it would serve to identify other classes of *E(top)* mutations.

Star is a known *E(top)* mutation on chromosome 2, producing *E(top)* phenotypes very similar to those of some of the *E(top)* mutations recovered. The fact that no alleles of *Star* were recovered in the screen suggests that the second chromosome has not been saturated for *E(top)* mutations. Thus, even amongst the subset of *E(top)*s that can be identified by virtue of their enhancement of the top^1 wing vein defects, the screens have not reached a level of saturation. This conclusion is also apparent from the fact that of the seven *E(top)* mutations identified on the third chromosome, only *Hairless* is

represented by two alleles.

The mutagen used in all four of the screens was EMS, which is the most effective mutagen for inducing large numbers of point mutations. If other mutagens were to be used, each mutagen would have its own bias with respect to the kind of mutation it would produce and might, therefore, produce mutations in other genes. The best approach would be to use P-elements to induce *E(top)* mutations. The use of P-element mutagenesis would facilitate the mapping, cloning and molecular characterization of any mutations identified.

Future Considerations.

For the second-chromosomal *E(top)*s, the obvious course of action is to obtain map positions for all 20 mutations; only then can the number of genes be established unequivocally. Several mutations warrant further study, especially those which were found to produce novel wing vein defects in their enhancement of the top^1 phenotype.

For the third-chromosomal *E(top)*s, additional research will be required to determine the roles of these mutations. The most intriguing possibility concerning the third-chromosomal *E(top)*s is to attempt to examine the possible relationship between the torpedo and Notch pathways.

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