Molecular Analysis of the Drosophila EGF Receptor Homolog Reveals That Several Genetically Defined Classes of Alleles Cluster in Subdomains of the Receptor Protein

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ABSTRACT

Mutations in the torpedo gene, which encodes the fruitfly homolog of the epidermal growth factor receptor (DER), disrupt a variety of developmental processes in Drosophila. These include the survival of certain embryonic ectodermal tissues, the proliferation of the imaginal discs, the morphogenesis of several adult ectodermal structures and oogenesis. torpedo is genetically complex: a number of alleles of the gene differentially affect the development of specific tissues, such as the eye, wing, bristles and ovary. In addition, torpedo mutations exhibit interallelic complementation. Molecular analysis of 24 loss-of-function mutations in the torpedo gene provides insights into the mechanistic basis of its genetic complexity. We observe an intriguing correlation between molecular lesions and mutant phenotypes. Alleles that differentially affect specific developmental processes encode receptors with altered extracellular domains. Alleles that fully or partially complement a wide range of embryonic and postembryonic torpedo mutations encode receptors with altered intracellular domains. From these findings we conclude the following. First, the torpedo protein may be activated by tissue-specific ligands. Second, the torpedo receptor tyrosine kinase may phosphorylate multiple substrates. Third, signal transduction by torpedo appears to require the physical association of receptors. Finally, the extracellular domain of the Torpedo protein may play an essential role in mediating receptor-receptor interactions.

RECEPTOR tyrosine kinases (RTKs) form a family of signal transduction molecules that has been conserved in metazoans for at least 800 million years. Tyrosine kinases that are structurally related to the epidermal growth factor receptor (EGF-R), for example, mediate cell-cell communication in organisms ranging from nematodes (AROIAN et al. 1990) to dipterans (LIVNEH et al. 1985; WADSWORTH et al. 1985) to humans (ULLRICH et al. 1984; COUSSENS et al. 1985; YAMAMOTO et al. 1986; KRAUS et al. 1989; PLOWMAN et al. 1990, 1993). The EGF-R protein-tyrosine kinase is a transmembrane protein with a single membrane-spanning domain. The extracellular (amino-terminal) portion of the receptor contains four subdomains (SI-SIV), of which SI and SIV are cysteine-rich, and SI and SIII are relatively cysteine-poor. The intracellular (carboxyl-terminal) portion of this protein consists of a short juxtamembrane domain, a tyrosine kinase domain and a carboxyl-terminal tail (see YARDEN and ULLRICH 1988).

Much of our understanding of the biochemistry of signal transduction by EGF-R and its relatives comes from vertebrate tissue culture studies (reviewed in ULLRICH and SCHLESSINGER 1990; SCHLESSINGER and ULLRICH 1992). This work has lead to a model for EGF-R function that is depicted in Figure 1. In the absence of ligand, EGF-R preferentially exists as a monomer. Upon ligand binding, which is mediated by the extracellular portion of the protein, the receptor forms dimers. Oligomerized receptors undergo phosphorylation on tyrosine residues located in the carboxyl terminus of the molecule by a trans-molecular mechanism. This autophosphorylation is thought to allow the specific binding of substrate and coupling molecules—via their SH2 domains—to phosphorylated tyrosine residues in the carboxyl-terminal portion of the receptor. Receptor activation involving autophosphorylation appears to promote receptor-substrate interactions and may stimulate substrate phosphorylation. The physical association with and/or phosphorylation of cellular proteins by EGF-R then initiates a biochemical cascade resulting in the transmission of a signal into the cell.

Cell culture studies have shed light on the role of EGF-R in the control of cell proliferation, differentiation and survival. Studies using ligands for EGF-R in organ culture and in whole organisms similarly have provided insights into the role of receptor function in specific tissues [see CLIFFORD and SCHÜPBACH (1992) for references]. Gene disruption provides another means of analyzing the role of this receptor in vertebrate development. This technique has been used to investigate the role of transforming growth factor-α, an activator of EGF-R (DERYNCK et al. 1984), in mouse development (LUETTEKE et al. 1993; MANN et al. 1993).
Studies in vertebrate systems have been complemented by the genetic analysis of receptor function in Drosophila and Caenorhabditis. The versatile genetics of the worm and fly facilitate examination of the developmental roles of these receptors through phenotypic analyses, the structure/function analysis of these molecules through molecular genetics, and the identification of additional components of the signalling pathway through the isolation of suppressor and enhancer mutations.

The torpedo gene encodes the Drosophila melanogaster homolog of the vertebrate epidermal growth factor receptor, DER (Price et al. 1989; Schejter and Shilo 1989). Over 40 mutant alleles of the gene, including both loss-of-function and two gain-of-function lesions, have been isolated (see Lindsley and Zimm 1992). Phenotypic characterization of these mutations has shown that the torpedo receptor tyrosine kinase performs multiple functions in Drosophila development.

In the embryo torpedo is necessary for the establishment of cell identity in the ventral ectoderm, the survival of amnioserosa and ventral ectodermal cells, the formation of the central nervous system, germ band retraction and the production of denticles, hooklike outgrowths of the ventral epidermis. Temperature-shift studies show that the torpedo product is required at several points in embryonic development (Clifford and Schüpbach 1992; Raz and Shilo 1992, 1993). torpedo does not appear to regulate the major phases of cell proliferation in the Drosophila embryo (Clifford and Schüpbach 1992).

During the larval and pupal stages of development, the torpedo product is needed in the imaginal discs for the proliferation or viability of cells. A partial loss of gene activity results in a severe size reduction in several imaginal discs (Clifford and Schüpbach 1989). Clones of cells lacking functional torpedo product are recovered in adult animals less frequently than clones of genetically wild-type cells. Moreover, mutant clones generally are smaller than wild type, which indicates that the growth of mutant tissue is impaired (Baker and Rubin 1989; Diaz-Benjumea and García-Bellido 1990; Xu and Rubin 1993; our unpublished observations). In the developing retina, gain-of-function mutations in torpedo lead to a great increase in the number of cells entering S phase (Baker and Rubin 1992; Zak and Shilo 1992), but few of these cells undergo mitosis (Baker and Rubin 1992).

torpedo is required in the pupa for imaginal disc morphogenesis. A variety of abnormalities are seen in the ectodermal tissues of adult flies suffering a partial loss of torpedo gene activity. These include roughened compound eyes, shrunken or missing ocelli, the deletion of certain wing veins, the duplication or elimination of sensory bristles and the elimination of tarsal claws (Clifford and Schüpbach 1989). Clones of ectodermal tissue possessing reduced torpedo activity show similar developmental abnormalities (Diaz-Benjumea and García-Bellido 1990).

If the torpedo gene encodes a single activity, it should be possible to arrange all alleles in a simple hypomorphic series progressing from weak to intermediate to complete loss-of-function mutations. Roughly half of the loss-of-function torpedo alleles, however, cannot be fit into such a series. These mutant alleles show complementation behavior consistent with the idea that they differentially affect specific gene functions. Certain lethal mutations fully or partially complement (Clifford and Schüpbach 1989; Raz et al. 1991), suggesting that the proteins encoded by these alleles are defective in different receptor activities. Other alleles appear to dif-

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**FIGURE 1.—Simplified model for signal transduction by receptor tyrosine kinases of the EGF receptor subfamily (adapted from Schlessinger and Ullrich 1992).** In the absence of ligand, the receptor chiefly exists as a monomer. (A) In the monomeric form, the receptor binds ligand with low affinity (thin arrow). (B) Ligand binding stimulates receptor dimerization. Ligand binding by one receptor may be sufficient for dimer formation (see discussion). (C) In the dimeric form, receptors display high affinity ligand binding (heavy arrow). (D) Dimerized receptors autophosphorylate on tyrosine residues located in the carboxyl-terminal domain of the molecule through a transmolecular mechanism. (E) Cellular substrates bind to specific phosphotyrosines via their SH2 domains. (F) Bound substrates are phosphorylated by the activated receptor.
Drosophila EGF Receptor Mutants

Developmental processes such as oogenesis and imaginal development of specific tissues (Clifford and Schüpbach 1989) differentially affect gene activities required for specific developmental processes such as oogenesis and imaginal disc patterning and/or morphogenesis (Baker and Rubin 1989; Clifford and Schüpbach 1989). The remaining mutations, in contrast, behave genetically as if they reduce all gene activities in a uniform fashion (Clifford and Schüpbach 1989).

To gain insight into the mechanistic basis of torpedo's genetic complexity, we have identified the presumptive molecular lesions of 24 loss-of-function alleles of torpedo. The examined mutations fall into four phenotypic categories. Class I alleles disrupt all gene activities in a uniform fashion, class II mutations are embryonic lethal lesions that fully or partially complement a variety of embryonic and pupal lethal torpedo alleles and the class III and class IV mutations differentially affect the development of specific tissues (Clifford and Schüpbach 1989). From this analysis we find a striking correlation between phenotypic classes and molecular lesions. Tissue-preferential alleles (with one exception) alter single amino acids in the extracellular domain of the receptor. The class II alleles, on the other hand, alter the intracellular portion of the molecule. We interpret the results of our molecular genetic study within the framework of a current biochemical model for EGF-R function.

MATERIALS AND METHODS

Nomenclature: Mutations in the gene encoding the Drosophila epidermal growth factor receptor homolog have been isolated independently by a number of workers. Consequently, the locus has been named Ellipse (Baker and Rubin 1989), faint little ball (Nüsslein-Volhard et al. 1984), torpedo (Schüpbach 1987) and l(2)57DEFa (O'Donnell et al. 1989). On the basis of the homology of its product to the vertebrate EGF receptor, the gene has been designated DER (Livneh et al. 1985), DEGFr (Dim-Benjumea and Garcia-Bellido 1990) and Egrf (Lindsley and Zimm 1992). In this work, we refer to all loss-of-function alleles of the gene as torpedo mutations, and the protein product of the locus as Torpedo.

The torpedo gene encodes two proteins which are approximately 90% identical and differ only at their amino termini (Schüpbach et al. 1986; Schüpbach and Súilo 1989) (Figure 2). Since all mutations characterized in this study affect residues common to both isoforms of the receptor, we refer to both protein products of the gene as Torpedo.

Stocks: The origins and phenotypes of torpedo mutations analyzed in this work, with the exception of topD, are described in Clifford and Schüpbach (1989). The topD allele was isolated in an X-ray mutagenesis by J. Price (Price et al. 1989); it is a weak zygotic embryonic lethal mutation that shows abnormally severe wing vein defects in combination with the viable mutation topF.

C. Situshkin kindly provided a chromosome containing the visible mutations straw, pawn, cinnabar and brown. The markers straw and pawn, which produce yellowish and truncated bristles, respectively, allow identification of clones in the cuticle (Garcia-Bellido and Dapena 1974). In a cn background, eye tissue that also is genotypically bw is completely unpigmented.

To maximize clone size, Minute" marked cells were induced in a M(2)em"/+ background. The dominant mutation M(2)em reduces the mitotic rate of cells; therefore cells within clones enjoy a growth advantage in the imaginal disc (Ferrus 1975; Morata and Ripoll 1975).

stw pawn cn bw sp, stw pawn cn topJ" bw sp and stw pawn cn topJ" bw sp mosaics were produced by irradiating 48 ± 12-hr-old progeny of stw pawn cn topJ" bw (sp)/CyO females mated to br cn M(2)E67/CyO males in a Torrent 150D X-ray machine (12 min at 145 kV and 5 mA, using an aluminum filter). Irradiated animals completed development at 25°C. stw pawn cn bw, stw pawn cn topJ" bw and stw pawn cn topJ" bw mosaic animals were generated by irradiation (1800 rad from a cobalt source at 36–60 hr of development). These irradiated animals completed development at 29°C.

stw pawn cn topJ" bw (sp)/br cn M(2)E67 flies were examined for eye clones under a dissecting microscope at 25×, then stored in 70% ethanol prior to mounting. Wings, heads and bodies were dissected apart, cleared with 10% NaOH and mounted in Faure's medium as described in Wieschaus and Nüsslein-Volhard (1986).

Isolation of genomic DNA: Total genomic DNA was prepared from embryos, larvae, pupae and adults essentially as described in Bender et al. (1983).

Polymerase chain reaction (PCR): Fragments of the torpedo gene were amplified by the polymerase chain reaction (Saiki et al. 1988) using the primers shown in Figure 2. DNA isolated from five embryos or 1/10 of an adult provided sufficient template for a typical PCR amplification. PCR buffer contained 50 mM KCl, 10 mM Tris (pH 8.3), 0.01% gelatin and either 1 mM or 2 mM MgCl₂. Amplification conditions for each genomic fragment are shown in Table 1.

Denaturing gradient gel electrophoresis (DGGE): Twenty prospective mutant alterations described in this work were identified and molecularly mapped through a DGGE. (Myers et al. 1985) protocol developed by M. Gray. Fragments of the

abnormalities observed for these alleles are due to dominant suppression by a plexus (px) mutation present on the topB1 and topB13 chromosomes. The px mutation, which produces excess wing veins, was previously shown by Diaz-Benjumea and Garcia-Bellido (1990) to enhance ectopic wing vein production by the gain-of-function torpedo mutation Ebf. Removal of px from the dp cn a topB1 px sp chromosome by recombination leads to a more severe wing vein phenotype in trans to topF. While 62% of L4 veins (n = 84) and 96% of anterior crossveins (n = 84) from cn topF bw/dp cn a topB1 bw animals were defective, 90% of L4 veins (n = 56) and 55% of anterior crossveins (n = 56) from cn topF bw/dp cn a topB1 px sp flies were gapped or missing.

Complementation analysis: Crosses were performed at room temperature (~22°C). Four to six topF/CyO or topB1/SIMI females were mated to an equal number of topF/CyO or varp/SIMI males. For embryonic lethal heteroallelic combinations, embryos were collected on apple juice agar plates as described in Wieschaus and Nüsslein-Volhard (1986). The trans-heterozygous phenotype, in most cases, is based on the examination of 50–150 dead embryos. For postembryonic lethal heteroallelic combinations, mutations were performed in vias. Mutant viability was calculated from the number of eclosing topF/CyO (or SIM1), topB1/CyO (or SIM1) and topB1/topB adults.

Mosaic analysis: To allow identification of torpedo and wild-type clones, we constructed mutant and control chromosomes marked with straw, pawn, cinnabar and brown. The markers straw and pawn, which produce yellowish and truncated bristles, respectively, allow identification of clones in the cuticle (Garcia-Bellido and Dapena 1974). In a cn background, eye tissue that also is genotypically bw is completely unpigmented.

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Figure 2.—The revised *torpedo* genomic sequence. The protein sequence is shown above the nucleotide sequence. Amino acid 1 of Torpedo corresponds to the initiation methionine for the type 2 receptor; residues specific to the type 1 receptor are not numbered. Signal peptide residues are italicized, cysteines in the extracellular domain are outlined, transmembrane domain residues are underlined and the tyrosine kinase domain is in bold italics. Nucleotide coordinates of the *torpedo* gene are based on LNNEH et al. (1985). Coding sequences are in upper case; noncoding and intron sequences are in lower case. Primer sequences are boldfaced, and the direction of the arrows indicates whether the primer corresponds to the coding or noncoding strand of the gene. Names of amplification primers are boldfaced; names of sequencing primers are in regular type. Three nucleotides absent from the published *torpedo* sequence are underlined.
Drosophila EGF Receptor Mutants

torpedo gene were amplified from genomic DNA by PCR. After phenol/chloroform and chloroform extraction, followed by ethanol precipitation, 1/10 of the product of the PCR amplification was cut with a restriction enzyme(s) to yield fragments approximately 800-800 base pairs in length. Digested mutant and control DNA samples were electrophoresed in polyacrylamide gels containing a gradient of the denaturants urea and formamide for 20 hr at 60° and 70 V, then visualized by ethidium bromide staining. For most mutants characterized, amplified fragments comprising 80% or more of the coding region were examined (Figure 3).

A physical mismatch within a melting domain of a duplex DNA molecule destabilizes the domain, thereby lowering its melting temperature. This property was exploited to increase the sensitivity of the electrophoretic screen. DNA fragments produced from heterozygous (mutant/balancer or mutant/
TABLE 1

PCR amplification conditions for genomic fragments

<table>
<thead>
<tr>
<th>Primers</th>
<th>[Mg&lt;sup&gt;2+&lt;/sup&gt;]</th>
<th>Duration (sec)</th>
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<tr>
<td></td>
<td>(mm)</td>
<td>Denaturation</td>
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<tr>
<td>15A-13A</td>
<td>1</td>
<td>30 (94&lt;sup&gt;°&lt;/sup&gt;)</td>
</tr>
<tr>
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<tr>
<td>755-1889</td>
<td>1</td>
<td>30 (94&lt;sup&gt;°&lt;/sup&gt;)</td>
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<tr>
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<td>1</td>
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<td>1</td>
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<tr>
<td>3212-4810</td>
<td>1</td>
<td>30 (94&lt;sup&gt;°&lt;/sup&gt;)</td>
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REVIEWED torpedo sequence: The genomic sequence of torpedo shown in Figure 2 is a revised version of the DER sequence submitted to GenBank (Luneh et al. 1985; Schechter and Shilo 1989). Nucleotides 1–652 of the published sequence, which encode the first common exon and its flanking sequences, have been replaced by nucleotides 251–692 (Schechter 1989). The identities of several ambiguous codons in the cytoplasmic portion of the molecule also have been resolved. Finally, three nucleotides (underlined in Figure 2) have been added to the region of the gene encoding the tyrosine kinase domain of the receptor. This last revision changes the identity of eight amino acids in subdomains VI and VII of Hanks et al. (1988) and adds a residue to subdomain VI; the updated protein sequence (. . . DLARNLVQGTPSLVKKITDFG . . .) more closely matches those of other members of the EGF-R subfamily of tyrosine kinases.

RESULTS

torpedo alleles show qualitative phenotypic differences: Genetic analysis has shown that many mutations in the torpedo gene differentially affect the development of tissues that require receptor activity. In a previous study (Clifford and Schüpbach 1989) we used interallelic complementation analysis to assay the degree to which a collection of loss-of-function alleles affect each developmental process. The mutations examined displayed qualitative as well as quantitative phenotypic differences in the affected tissues. From this work, we concluded that torpedo encodes several differentially, but not independently, mutable activities. On the basis of their complementation behavior, we have divided the 24 torpedo alleles examined in this study into four functional categories (Table 2). These categories largely correspond to the four phenotypic classes defined in our earlier genetic studies.

The 12 class I lesions appear to disrupt all gene activities in a uniform manner. Alleles of this category range in severity from the partial loss-of-function, adult viable mutation top<sup>1</sup> to the complete loss-of-function, embryonic lethal allele top<sup>K35</sup>. All homozygous lethal class I mutations enhance the female sterile and adult morphological defects of top<sup>1</sup> and rarely survive in combination with the semiviable allele top<sup>CA</sup>.

The class II mutations, in contrast, are embryonic lethal lesions that fully or partially complement the developmental defects of top<sup>1</sup> and top<sup>CA</sup>. These four alleles also substantially complement the postembryonic lethality of the pupal lethal lesion top<sup>C10</sup>. The class IIA alleles top<sup>01</sup>, top<sup>23G4A</sup> and top<sup>24G5</sup> more fully complement top<sup>1</sup>, top<sup>CA</sup> and top<sup>C10</sup> than does the class IIB allele top<sup>2X1</sup>, which was previously designated a class IV mutation.

The complementation behavior of the class III and IV torpedo mutations is consistent with their differentially affecting a subset of tissue-specific gene functions. The class III lesions top<sup>K12</sup> and top<sup>38</sup> enhance the oogenesis defects of viable alleles to a lesser extent than do class I mutations of equivalent severity. The six class IV mutations show more severe or less severe adult morphological defects in combination with top<sup>1</sup> than do class I alleles exhibiting an equivalent homozygous phenotype. In trans to a viable allele, top<sup>CA</sup> and top<sup>38</sup> show unusually severe bristle defects, top<sup>1</sup> unexpectedly severe wing vein abnormalities, top<sup>CA</sup> and top<sup>38</sup> unusually weak eye defects and top<sup>ED16</sup> abnormally severe eye defects.

Further genetic analysis has lead us to reclassify a second torpedo mutation examined in this study. The class I allele top<sup>R1</sup> was originally placed in class IV because it exhibits mild wing vein defects in trans to viable alleles. We now have evidence that the weak wing venation ab-
normalities observed for this alleles result from dominant suppression by the plexus mutation present on the mutant chromosome rather than an intrinsic property of the top allele itself (see MATERIALS AND METHODS).

Interallelic complementation: We have extended our previous genetic analysis of torpedo by performing pairwise complementation tests between 20 of the alleles molecularly characterized in this study. Examples of positive complementation, in which the phenotype of the trans-heterozygote is less severe than that of either homozygote, and instances of weak negative complementation, in which the heterozygous phenotype is more severe than anticipated, were seen (Figure 4). Some of these genetic interactions were also described by RAZ et al. (1991). The observed pattern of interallelic complementation argues that the physical association of receptors is essential for signal transduction by torpedo (see DISCUSSION).

Most instances of positive complementation involve four embryonic lethal mutations: the class IIA alleles top101, top2c82 and top2165, and the class IIB allele top2X51 (Figure 4). All four mutations survive in trans to the postembryonic class I lethal allele topE20-10 to 50% of the trans-heterozygotes survive to adulthood, while the remainder die as pupae. The class IIA alleles are semi-viable in combination with the class IV pupal lethal mutations top9E8 and top9E10. Class IIA mutations, in addition, partially suppress the embryonic lethal phenotypes of the class I alleles top2R16, top2w74, top2b92 and top2f11. For example, while head morphogenesis and/or germband retraction abnormalities are seen in roughly 80% of top2f26 homozygotes and 60% of top2w74 homozygotes, we observe these defects in less than 5% and 30% of top2f26/top2f26 and top2w74/top2w74 heterozygotes, respectively (Figure 4; data not shown). Likewise, more than 90% of animals homozygous for top2f26, top2b92 or top2f11 show a severe embryonic lethal phenotype, but more than 80% of top2f26/top2f26 and top2w74/top2w74 trans-heterozygotes display a significantly milder phenotype (Figures 4 and 5, E–G; data not shown). Positive complementation between top2w74 and the class IIA alleles top2c82 and top2165 was also seen by RAZ et al. (1991). Further, all three Class IIA lesions partially complement the severe class IIB mutation top2X51 (Figure 4). The moderate Class IIA mutation top2c82 and the severe class IIA allele top2165 show weak embryonic lethal phenotypes in trans to top2X51 (see also RAZ et al. 1991). Some top9E8/top2X51 heterozygotes even survive to adulthood.
Striking positive complementation is observed between certain trans-heterozygous combinations of class IIA mutations. The weak embryonic lethal mutation top191 fully complements the moderate embryonic lethal mutation top265 and the severe embryonic lethal mutation top160/top265 and top160/top263 animals are completely viable, morphologically normal and fertile (Figure 4, data not shown).

Certain class I and class IV postembryonic lethal alleles also exhibit positive genetic interactions with one another. The degree of complementation between these mutations, however, is less complete than that occurring between class IIA mutations. For example, top265/top263 trans-heterozygotes usually survive to adulthood, but exhibit eye, wing and bristle abnormalities (Figure 4, data not shown).

We also observed examples of negative complementation. The pupal lethal class IV alleles top19 and top26, for example, survive in combination with the class IV mutation top116 less frequently than do the class I embryonic lethal alleles top160 and top263 (Figure 4; CLIFFORD and SCHÜPBACH 1989). The class IV mutation top116 appears to show weak negative complementation in combination with a variety of alleles (Figure 4). In their genetic analysis, RAZ et al. (1991) observed negative complementation between the moderate embryonic lethal mutation flb93 (not examined in this study) and top1793, top1841, top2587 and top3951.

We also observe differences in the complementation behavior of alleles showing similar homozygous phenotypes. The top19, top126 and top284 animals show similar weak embryonic lethal phenotypes at room temperature. Yet, in combination with top284, top284 and top128, the phenotype of top19 is enhanced so that roughly 25% of the heterozygotes show a moderate or severe embryonic lethal phenotype. In contrast, fewer than 5% of animals homozygous for top126 or top284 and top284 show these phenotypes. In fact, the embryonic lethal phenotypes of top126 and top284 are weakly suppressed by top284 (Figure 4, data not shown).

**Class IIA alleles do not disrupt tissue-specific gene functions**: Since the class IIA mutations fully complement the pupal lethality of top26 and top263, we proposed that they might preferentially disrupt a function of the torpedo gene required specifically for embryogenesis (CLIFFORD and SCHÜPBACH 1989). Under this hypothesis, the viability of animals trans-heterozygous for a class IIA allele and a pupal lethal mutation would result from the pupal lethal allele supplying normal gene function during embryogenesis and the class IIA allele supplying gene function during postembryonic development. If this were the case, we would expect class IIA lesions to
complement the postembryonic lethality of all pupal lethal *torpedo* alleles and to show mild adult morphological abnormalities in combination with these mutations. Further, if the class IIA alleles primarily affect receptor function in the embryo, cells homozygous for these alleles should develop more-or-less normally as long as homozygosity is induced after embryogenesis.

To address this possibility we examined the phenotypes of the class IIA alleles *top*"A1, *top"C82 and *top"31.65 in combination with the pupal lethal alleles *top"B8 and *top"2D16, which are more severe loss-of-function mutations than either *top"CA or *top"C26. All three alleles show reduced viability in trans to *top"B8 and *top"2D16 (Figure 4); furthermore, heterozygous animals that survive to adulthood exhibit severe eye, wing and bristle defects (data not shown). These results argue that *top"A1, *top"C82 and *top"31.65 disrupt the development of imaginal tissue.

In addition, we have shown that the class IIA mutations *top"C82 and *top"31.65 strongly disrupt the development of imaginal tissues using genetic mosaic techniques (see MATERIALS AND METHODS). Clones of cells homozygous for *top"C82 and *top"31.65 were recovered in the compound eye at a lower frequency than wild-type or heterozygous flies contained small scars similar to those seen in irradiated animals homozygous for the amorphic class I allele *top"C0. These scars are likely to result from the abnormal development of cells in a mutant clone (Table 3, data not shown). Likewise, *top"C82 and *top"31.65 clones in the leg–like *top"C0 clones—were recovered less frequently than genetically wild-type clones (Table 3). Mutant leg clones were also smaller than wild type. While most wild-type clones in the leg consisted of at least 50 bristles, *top"C82 and *top"31.65 mosaic legs contained fewer than 10 marked bristles (data not shown). Mosaic analysis therefore indicates that *top"C82 and *top"31.65 impair the development of imaginal disc cells and behave as severe loss-of-function alleles in the imaginal discs.

These experiments show that the class IIA alleles *top"A1, *top"C82 and *top"31.65 do not preferentially disrupt embryogenesis. Instead, the receptors encoded by these alleles are not fully functional in any of the various tissues examined. Therefore, the extensive complementation of pupal lethal alleles displayed by these class IIA mutations may result from their products interacting with other mutant receptors to transduce signals. Complementation between class IIA and pupal lethal alleles appears to occur in embryonic as well as in imaginal tissues, *top"2D16, a pupal lethal allele complemented by the class IIA mutations, must be defective for a gene function required for embryonic development, since animals trans-heterozygous for *top"2D16 and any of a number of embryonic lethal mutations die during embryogenesis (Figure 4).

**Sequence analysis of torpedo alleles:** Genetic analysis reveals qualitative differences between *torpedo* alleles
and a complex pattern of interallelic complementation. To gain insight into the mechanistic basis of torpedo's genetic complexity we have performed a molecular analysis of mutant alleles. Nucleotide alterations associated with 24 loss-of-function mutations in the torpedo gene, which represent each of the four phenotypic classes, have been identified (see MATERIALS AND METHODS). Five mutations described below, top^{IF26}, top^{2C82}, top^{2L65}, top^{2W74} and top^{2X51}, were also characterized by Raz et al. (1991).

Nonsense mutations: Five alleles characterized in this study are nonsense mutations (Table 2, Figure 6). The predicted proteins encoded by top^{1835}, top^{2631}, top^{38} and top^{EE10} terminate within the extracellular domain of the receptor; these putative secreted molecules lack the bulk of the ligand binding domain, as well as the entire transmembrane, tyrosine kinase and carboxy-terminal domains. The top^{1PO2} product, on the other hand, is truncated within the tyrosine kinase domain of the receptor, resulting in the deletion of kinase subdomains X and XI of Hanks et al. (1988) and the carboxyl-terminal tail of the protein.

While most embryos homozygous for each of the nonsense mutations show a severe zygotic lethal phenotype, a fraction of top^{1PO2}, top^{2C31} and top^{EE10} animals—10, 15 and 11%, respectively—exhibit a somewhat weaker
terminal phenotype (Table 4). However, even embryos homozygous for the deletion allele top18A occasionally exhibit a moderate lethal phenotype (Table 4). It therefore seems likely that genetic background affects the final cuticle morphology of the mutant embryo. It is also possible that the top18P0Z, topP3G3 and topEP39 nonsense mutations are leaky.

Genetic background variation does not appear to be sufficient to explain the phenotype of the nonsense mutation top18. Animals homozygous for this exceptional allele are 2-fold more likely than embryos homozygous for any other nonsense mutation, and 10-fold more likely than null embryos, to exhibit a moderate phenotype. Ten percent of top18/top18 embryos, in fact, exhibit an embryonic lethal phenotype characterized by the production of essentially wild-type amounts of ventral cuticle (Table 4, Figure 5H). The partial loss-of-function character of top18 may be explained in several ways. One possibility is suppression by a second-site lesion that is linked to top18. Alternatively, the hypomorphic behavior of top18 could result from readthrough of its amber codon or from translational reinitiation at a downstream methionine codon.

Deletion mutation: Nine contiguous nucleotides are deleted from the topED26 chromosome, resulting in the loss of three amino acids located in subdomain III of the ligand binding domain of the protein (Table 2, Figure 6). Given the amorphic phenotype of topED26 (Table 4), the three residues eliminated by this mutation appear to be essential for receptor function. Biochemical studies of the human EGF receptor implicate subdomain III of the molecule in receptor-ligand interactions (Lax et al. 1989, 1991; Wu et al. 1990). The portion of the ligand binding domain deleted by the topED26 lesion is poorly conserved among receptor tyrosine kinases of the EGF-R subfamily (Figure 7G); such variability might be expected of a region of the molecule that is specialized for interaction with a specific ligand or set of ligands. It is possible, however, that the TopED26 receptor is defective in receptor-receptor interactions or stability.

Frameshift mutation: In the top2X51 chromosome, a thymidine residue at genomic position 3989 is replaced by guanidine and adenine (Table 2, see also Raz et al. 1991). The protein encoded by this frameshift allele lacks most of its carboxyl-terminal tail (Figure 6), which includes tyrosine residues thought to mediate receptor-substrate interactions (reviewed in Schlessinger and Ullrich 1992). The carboxyl terminus of Torpedo is essential for receptor activity, as top2X51 homozygotes show a severe loss-of-function phenotype (Table 4). Despite this defect, Top2X51 does appear to be able to form functional heterodimers with other mutant receptors (Figure 4). For a discussion of this phenomenon, see Raz et al. (1991).

Missense mutations: Nucleotide alterations associated with 17 torpedo mutations lead to amino acid substitutions. Three of these amino acid replacements severely disrupt receptor activity, while the remainder result in a partial reduction of protein function.

Extracellular domain mutants: Twelve missense mutations alter residues lying within the ligand binding domain of the Torpedo protein. Five of these affect amino acids located in the relatively cysteine-poor subdomains I and III, which, in the case of the human EGF receptor, contain determinants of ligand binding (Lax et al. 1989, 1991). The viable top1 and topP3 mutations, which were isolated independently but show similar phenotypes, contain the same amino acid alteration. The two alleles were induced on different parental chromosomes that can be distinguished by their characteristic sequence polymorphisms (Figure 8). In both cases, a non-conserved serine residue in subdomain I is changed to phenylalanine (Table 2, Figures 6, 7A and 8). The nucleotide alteration associated with the severe lesion topB1 results in a Ser to Phe substitution in a highly variable region of extracellular subdomain 1 (Table 2, Figures 6 and 7B). Chemical cross-linking studies indicate that the homologous region of HER is in close proximity to the ligand (Woltjer et al. 1992). The T to A substitution associated with the weak embryonic lethal allele topP12 leads to the replacement of a Leu residue five amino acids carboxyl-terminal to the serine affected by the topB1 mutation with Gln. This portion of the molecule may form an amphipathic α-helix, as all receptors of the subfamily show a conserved spacing of hydrophobic residues separated by hydrophilic amino acids (Ala/Val/Met-X-X-Leu/Val-X-Leu/Met/Ile-X-X-Leu-X-Ile/Val). The second hydrophobic residue of this sequence is altered in the TopB1 protein (Table 2, Figures 6 and 7B).
The nucleotide alteration associated with the partial loss-of-function, pupal lethal mutation topC20 produces an Asp to Asn substitution in subdomain III of the ligand binding domain (Table 2, Figures 6 and 7). Although the affected residue is not conserved among members of the EGF-R subfamily, most proteins in the subfamily do possess at least one negatively charged amino acid between the conserved Gly and Phe residues (Figure 7F).
**Drosophila EGF Receptor Mutants**

**Figure 7.** Conservation of extracellular domain amino acids affected by missense and deletion mutations. Aligned protein sequences for the human epidermal growth factor receptor (HER) (ULLRICH et al. 1984), HER2 (COUSSENS et al. 1985), p160erbB3 (HER3) (US et al. 1989), and p180erbB4 (HER4) (PLOW et al. 1993) receptor tyrosine kinases, the melanoma-associated receptor tyrosine kinase of Xiphophorus (Xmrk) (WITTBRODT et al. 1989), the Zet-23 receptor tyrosine kinase of *Caenorhabditis elegans* (Let23) (HORN et al. 1990) and the torpedo receptor tyrosine kinase of *D. melanogaster* (Top) (LIVNEH et al. 1985; this work). *, invariant residue; +, amino acid conserved among at least five proteins. Conservative amino acids: D/E F/Y; I/L/M/V; H/K/R; N/Q; S/T. Torpedo residues altered by missense and deletion lesions are shown below the aligned sequences.
Seven mutations affect residues within the cysteine-rich repeats of the protein. In the human EGF receptor, these subdomains (II and IV) appear to serve as structural elements of the ligand binding domain (LAX et al., 1989, 1991). The two most severe mutations in this group change cysteine residues to serine. The product of top, a severe embryonic lethal allele, lacks a cysteine residue in subdomain II; the receptor encoded by top, a weak embryonic lethal allele, lacks a cysteine located in subdomain IVB of the ligand binding domain (Table 2, Figures 6 and 7, E and I). Nucleotide alterations associated with the weakest mutations in this group, top (viable), top (semilethal), top (late embryonic lethal), top (pupal lethal) and top (pupal lethal), alter non-cysteine residues in subdomains II and IV. In the Top and Top proteins, charged residues are changed to hydrophobic or oppositely charged amino acids (Table 2, Figures 6 and 7, C and E); in Top, a charged residue is replaced by cysteine (Table 2, Figures 6 and 7D); in Top and Top, charged amino acids substitute for glycines (Table 2, Figures 6 and 7, H and J). Charged residues, which can form salt bridges, and glycines, which provide flexibility to the polypeptide chain, may play important roles in maintaining protein structure. The relative severity of mutations in the cysteine-rich motifs is consistent with the idea that disulfide bonding plays a central role, and ionic interactions a more peripheral role, in maintaining the structure of the extracellular domain.

**Tyrosine kinase domain mutants:** Nucleotide changes associated with five missense mutations lead to the alteration of residues in the kinase domain of Torpedo. The recent determination of the crystal structure of the catalytic subunit of murine cAMP-dependent protein kinase has revealed that the catalytic core domain of a protein kinase is composed of two lobes. Amino-terminal residues of the kinase domain form the smaller lobe, which is comprised of two β-sheets and two α-helices; carboxyl-terminal residues of the domain form the larger lobe, which contains two β-sheets and seven α helices (KRIGHTON et al. 1991). All of the kinase domain mutations affect residues located within the large lobe of the domain, between β-strand 9 and α-helix H (Figure 9), a portion of the kinase domain that contacts substrate molecules.

The nucleotide alteration associated with the weak embryonic lethal allele top() mutation produces a conservative Pro to Ser change in the loop connecting β-strand 9 to α-helix F of the kinase domain (Table 2, Figures 6 and 9). This amino acid lies in a portion of the tyrosine kinase domain proposed to act as a determinant of protein kinase specificity. In this region of the kinase domain, the consensus sequence for protein-tyrosine kinases is X-Pro-Ile/Val-Lys/Arg-Trp-Thr/Meta-Ala-Pro-Glu, while that for serine/threonine kinases is Gly-Thr/Ser-X-X-Tyr/Phe-X-Ala-Pro-Glu (HANKS et al. 1988). The Let-23 protein-tyrosine kinase, however, possesses Ala, rather than Pro, at this position (AROMAN et al. 1990).

In the Top protein, Ile replaces Thr, a highly conserved residue situated at the carboxyl-terminal end of helix F (Table 2, Figures 6 and 9). This amino acid change generates a contiguous stretch of four hydrophobic residues with fairly extensive side chains (Leu-Leu-Ile-Phe); such a sequence may not assume a stable α-helical conformation.

The remaining three kinase domain mutants map to the loop connecting helix G and helix H. In the product of the moderate embryonic lethal mutation top, the Gly residue just carboxyl-terminal to helix G is changed to Ser. In the Top receptor, proline, a residue in the loop connecting helices G and H, is replaced by the nonconservative amino acid leucine. The product of the severe zygotic embryonic lethal allele top() suffers a Ser to Leu substitution at the residue abutting, or within, the amino terminus of helix H (Table 2, Figs. 6 and 9). The serine altered in Top is less highly conserved than the residues affected by other kinase domain mutations, as alanine, proline, serine and threonine can be found at this position in tyrosine kinases (HANKS et al. 1988).

**Temperature-sensitive mutations:** Three of the missense mutations, top, top, and top, are temperature-sensitive. Animals homozygous for these alleles show a severe embryonic lethal phenotype at 29° and a

The nucleotide change associated with the top$^{SH2}$ mutation alters a leucine in subdomain I of the ligand binding domain to the polar residue glutamine. The affected amino acid may lie within an amphipathic α-helix (see above).

In the product of the top$^{2WT4}$ allele, a polar amino acid in helix F of the kinase domain is replaced by a hydrophobic residue. The conditional allele sec$^{A2}$ contains a homologous alteration which changes the peptide sequence Ile-Leu-Thr-Leu to Ile-Leu-Ile-Leu; Mullins and Rubin (1991) provide evidence that this mutant Sevenless protein is temperature-sensitive for activity, rather than synthesis. This alteration could produce a temperature-sensitive product in a number of ways. Perhaps the bulky, relatively inflexible side chain of isoleucine produces steric hindrance resulting in the destabilization of the α-helix. This instability might worsen with increasing temperature. Another possibility is that the isoleucine residue participates in hydrophobic interactions with neighboring nonpolar amino acids. The strength of these novel interactions may be greater at higher temperatures.

The nucleotide alteration in the top$^{I276}$ chromosome leads to the substitution of Leu for Pro in the random coil linking helices G and H of the tyrosine kinase domain. There appears to be a strict requirement for a proline within this portion of the kinase domain, as every protein kinase in the database of Hanks et al. (1988) possesses at least one proline within two residues of Pro$^{113}$. Loss of this amino acid could increase the flexibility of the loop, a defect that might become more extreme at higher temperatures.

**Correlation between amino acid changes and mutant phenotypes:** We observe a striking nonrandom distribution of lesions within the gene. Mutations producing a general disruption of gene functions (class I) are scattered throughout the coding region of torpedo, while those affecting a subset of gene functions (classes II, III and IV) are clustered within specific functional domains of the Torpedo protein (Figure 6).

Class I mutations appear to reduce the activity of the Torpedo protein in all tissues. A variety of biochemical defects (ligand binding, receptor dimerization, tyrosine kinase activity, substrate interaction) could lead to a general disruption of receptor activity. Likewise, altering residues at many locations in the protein could lead to defects in stability, post-translational processing or transport to the membrane. Consistent with this expectation, we find that some class I lesions alter amino acids located in the extracellular domains while others affect amino acids lying in the cytoplasmic domain of Torpedo.

The class IIA mutations analyzed in this study, top$^{101}$, top$^{206}$ and top$^{216}$, may disrupt a subset of receptor activities required in every tissue (see DISCUSSION). The nucleotide changes associated with these alleles alter amino acids lying in the portion of the tyrosine kinase domain that physically interacts with substrates; thus it is possible that the Top$^{101}$, Top$^{206}$ and Top$^{216}$ proteins are unable to phosphorylate one or more ubiquitous substrates. Receptor autophosphorylation sites, as well as cellular proteins, could serve as these substrates. The class IIB allele top$^{2X31}$ encodes a receptor lacking a carboxyl-terminal domain. While this molecule should possess kinase activity, it should not be able to physically associate with SH2 domain-containing cellular proteins.

The product of the class III allele top$^{SH2}$ carries an amino acid substitution in subdomain I of the ligand
binding portion of the receptor. Chemical cross-linking studies with the human epidermal growth factor receptor show that bound EGF lies in close physical proximity to this portion of the molecule, which suggests the intriguing possibility that the \( \text{top}^{\text{SH2}} \) missense mutation alters a region of Torpedo involved in ligand binding. The class III mutation \( \text{top}^{\text{E3}} \), on the other hand, is a nonsense mutation located in the extracellular domain of the protein that exhibits a partial loss-of-function phenotype (Table 4). The weak female sterile phenotype of this mutation in \( \text{trans to top}^1 \) might indicate that the mechanism by which this mutation is suppressed operates more efficiently in the ovary than in other tissues.

All class IV alleles are missense mutations that produce amino acid substitutions in the ligand binding domain of the receptor (Figure 6). \( \text{top}^{\text{E2A}}, \text{top}^{\text{E3A}}, \text{top}^{\text{E8}}, \text{top}^{\text{E16}} \), and \( \text{top}^{\text{E33}} \) differentially affect the development of adult ectodermal structures, the eye, wing veins and bristles. The molecular nature of these mutations suggests the possibility that their products may be preferentially impaired in binding tissue-specific ligands. As \( \text{top}^{\text{E4}} \) shows a slightly antimorphic phenotype in the wing (data not shown), its product may not dimerize properly.

**DISCUSSION**

Mutations in the Torpedo gene show a complex pattern of interallelic complementation. Based on genetic studies, we have divided the alleles into four phenotypic classes. Molecular analysis reveals that mutations of certain classes cluster within particular subdomains of the receptor. Class II mutations, embryonic lethal lesions that completely or partially complement a variety of embryonic and pupal lethal torpEd alleles, map to the portion of the gene encoding the extracellular domain of the Torpedo protein. The class IV mutations differentially affect the development of specific adult tissues, such as the eye, wing veins or bristles. These tissue-preferential alleles alter single amino acids in the extracellular domain of the receptor. Likewise, the class III mutation \( \text{top}^{\text{SH2}} \), which differentially affects oogenesis, changes a residue in the extracellular domain of Torpedo.

Do the identified molecular alterations correspond to the mutant lesions? Given the large coding region of Torpedo (\( \sim 4.4 \text{ kb} \)), we chose to search for mutant lesions by DGGE rather than by sequencing the entire gene. For this reason, we cannot rule out the possibility that some mutant chromosomes contain molecular lesions in addition to the ones identified. Nonetheless, we believe that the relevant DNA alterations have been identified in most cases for the following reasons. First, only a single PCR fragment amplified from each mutant chromosome showed a mobility shift in the DGGE assay (80% or more of the Torpedo coding region was typically examined) (Figure 3). Second, nucleotide alterations associated with the Torpedo alleles are consistent with their mode of generation. Molecular characterization of a large collection of lesions induced at the rosy locus by ethyl methanesulfate (EMS) or ethylnitrosurea (ENU) (summarized in Lindsley and Zimm 1992) has shown that these alkylating agents usually generate G/C to A/T substitutions. Nineteen of twenty-three nucleotide alterations detected in chromosomes carrying EMS- or ENU-induced \( \text{torpedo} \) mutations are G to A transitions (Table 2). Third, studies in which EMS-induced mutations in the sevenless (Mullins and Rubin 1991), Toll (Schneider et al. 1991) and dorsal (Isoda et al. 1992) loci were identified by sequencing the entire coding region of the gene indicate that double hits are infrequent. Twenty-seven of 29 alleles characterized in these studies possess single molecular alterations.

In addition, the biochemical properties of certain mutant gene products are consistent with their proposed molecular structure. The receptors encoded by \( \text{top}^{\text{E1}} \) and \( \text{top}^{\text{E23}} \), which are predicted to carry missense alterations in their extracellular domains, possess kinase activity (Scheijter and Shilo 1989). The Top\(^{\text{K18}}\), Top\(^{\text{I92}}\), Top\(^{\text{G31}}\) and Top\(^{\text{E35}}\) proteins, which are predicted to lack autophosphorylation sites, are kinase- in both in vivo and in vitro assays.

Further, the locations of the \( \text{top}^1 \) and \( \text{top}^{\text{E4}} \) mutant lesions are confirmed by genetics. A wild-type chromosome produced by a recombination event between the \( \text{top}^1 \) and \( \text{top}^{\text{E4}} \) mutations (J. Price and T. Schüpbach, unpublished) carries a sequence polymorphism specific to \( \text{top}^{\text{E4}} \) at nucleotide 401 and a polymorphism specific to \( \text{top}^1 \) at nucleotide 1545 (data not shown).

Alleles that differentially affect the development of specific tissues encode receptors with altered extracellular domains: Eight alleles examined in this study show tissue-preferential effects. These mutations show complementation behavior consistent with the idea that they preferentially disrupt a subset of torpedo-dependent developmental processes (see above). Seven of these alleles encode receptors carrying amino acid substitutions in their extracellular domains (Table 2). Several mechanisms can account for the genetic behavior of these mutations. One possibility is that the amino acid alterations resulting from the class III and IV missense mutations destabilize the Torpedo protein in a tissue-preferential fashion.

A second is that alterations in the extracellular domain of Torpedo differentially affect the development of certain tissues by disrupting different aspects of receptor dimerization. If the kinetics of signal transduction differ among tissues, mutations decreasing the rate of dimer formation could preferentially disrupt the development of one cell type, while those disrupting the maintenance of stable dimers could preferentially disrupt the development of another cell type.

A third possibility is that amino acid substitution in the extracellular domain of the receptor preferentially disrupt the binding of tissue-specific ligands. Indeed, Torpedo does appear to be activated by tissue-specific
ligands, gurken, a gene that exclusively regulates Droso-
phila oogenesis, is required in germ cells and acts up-
stream of torpedo in the signalling pathway (Schüpbach
1987). Gurken encodes a molecule showing similarity
to transforming growth factor-α (TGF-α) (Neuman-
Silberberg and Schüpbach 1993), a ligand for EGF-R
(Derynck et al. 1990), and thus may represent a
germline-specific ligand for Torpedo. The product of
the spitz gene of Drosophila, which also encodes a
TGF-α homolog (Rutledge et al. 1992), may represent
another ligand for Torpedo. Animals that lack zygotic
spitz expression die during embryogenesis and show a
subset of the defects seen in severe torpedo mutants
(Mayer and Nüsslein-Volhard 1988; Raz and Shilo
1992; Rutledge et al. 1992). The three mechanisms are
not mutually exclusive.

Torpedo may phosphorylate multiple substrates:
The most dramatic examples of complementation be-
tween mutant receptors involve the products of the class
IIA embryonic lethal torpedo alleles: top^{101}/top^{202A}
and top^{101}/top^{216S} trans-heterozygotes are indistinguish-
able from genetically wild-type animals (Figure 4). Since
the molecular lesions associated with these three alleles alter
coding for amino acids in a region of the kinase
domain that is likely to contact receptor substrates
(Knighton et al. 1991), we favor the idea that these mu-
tant receptors are impaired in their ability to interact
with substrate molecules. Because top^{101}, top^{202A}
and top^{216S} disrupt the development of every tissue tested,
we propose that their products are defective in interacting
with ubiquitous cellular substrates (either cytosolic pro-
teins or specific tyrosine residues in the carboxyl-
terminal domain of Torpedo itself). Complementation
between Top^{101} and Top^{216S}, for example, would occur
because the two mutant receptors fail to interact with
different sets of substrates. The lack of complementation
between Top^{202A} and Top^{216S}, on the other hand,
would result from the inability of both mutant receptors
to interact with one or more common substrates.

The class I proteins Top^{1926} and Top^{2674} partially
complement Top^{101}, Top^{202A} and Top^{216S} (Figure 4).
Since the residual activity possessed by these class I pro-
teins allows only weak complementation of Top^{101},
Top^{202A} and Top^{216S}, we favor the idea that the amino
codons producing by top^{202A} and top^{2674} impair
receptor interactions with all substrates.

Other mechanisms can account for the complement-
tion occurring between alleles that encode receptors
with altered kinase domains. One alternative is that
complementation reflects the mutual stabilization of
mutant receptors. Another is that receptor-receptor,
receptor-substrate or even receptor-ligand interactions
that are defective in homodimers are stable in het-
erodimers. Further studies are required to determine
which of these possibilities is correct.

Signal transduction by torpedo may require the physi-
ological interaction of receptors: Biochemical studies of
the human EGF receptor suggest that receptor
oligomerization-stimulated by ligand binding—is essen-
tial for signal transduction by this molecule (Figure 1;
reviewed in Ullrich and Schlessinger 1990). The ex-
tracellular domain of EGF-R plays a dual role in receptor
dimerization. This portion of the molecule contains not
only determinants of ligand binding (Lax et al. 1989,
1991; Wu et al. 1990; Wolter et al. 1992) but also de-
determinants mediating the physical association of recep-
tors (Smiraglia-Krollman et al. 1992).

The pattern of complementation between torpedo al-
leles is consistent with the idea that signal transduc-
tion by Torpedo depends upon the physical association
of receptors. In several cases partial complementation is
observed between a Torpedo molecule with an altered
extracellular domain but normal tyrosine kinase and
carboxyl-terminal domains (abbreviated as lig^{-}kin^{-} ter^{-} in
Table 2) and a Torpedo protein with an altered ty-
rosine kinase domain but normal extracellular and
carboxyl-terminal domains (abbreviated as lig^{+} kin^{-} ter^{+} in
Table 2). Top^{1926} and Top^{216S} represent one such pair
of mutant receptors. A simple interpretation of this
complementation is that mutant receptors form a
functional heterodimer. In this model, formation of the
heterodimer would be mediated by the intact extracel-
lular domain of the lig^{-} kin^{-} ter^{-} receptor and activation
of the receptor complex would be mediated by the intact
extracellular domain of the lig^{+} kin^{-} ter^{+} receptor.

If the biochemical defect of a kin^{-} receptor could be
complemented simply by the expression of a kin^{+} mole-
cule in the same cell, then all Torpedo proteins posses-
sing an intact tyrosine kinase domain should comple-
ment a given kinase-impaired receptor equally well. This
is not the case (Figure 4). Instead we find that the ca-
cacity of a kinase impaired molecule, such as Top^{2165},
to form an active signal transduction complex with a lig^{+}
kin^{-} ter^{+} Torpedo protein is roughly inversely propor-
tional to the severity of the lesion in the extracellular
domain, such that receptors with the most severely af-
fected extracellular domains (as judged by homozygous
mutant phenotypes) show the weakest complementa-
tion with lig^{+} kin^{-} ter^{+} proteins. This finding is consis-
tent with the idea that intermolecular complementation
depends upon the physical association of mutation re-
cessors and is mediated through the extracellular
domain of the molecule.

The pattern of interallelic complementation argues
that the extracellular domain of Torpedo plays a critical
role in mediating complementation between mutant re-
cessors. This requirement is illustrated by the positive
complementation behavior of the class II alleles, whose
products appear to possess normal ligand binding and
carboxyl-terminal domains and an altered tyrosine ki-
nase domain. While the lig^{+} kin^{-} ter^{+} product of the
severe class IIA allele top^{2165} fully complements the lig^{+}
kin^{-} ter^{+} molecule encoded by the weak embryonic le-
th class IIA mutation top^{101}, it only partially comple-
ments the pupal lethal alleles top^{E2} and top^{E14}, which
encode lig^- kin^- ter^+ receptors (Figure 4). Likewise,
top^{245} is complemented more fully by the severe em-
byronic lethal mutation top^{251} (lig^- kin^- ter^+ product)
that by the severe embryonic lethal alleles top^{392} and
top^{E1} (lig^- kin^- ter^+ products) (Figure 4).

Further evidence that the ligand binding domain of
Torpedo is required for complementation between mut-
ant receptors is provided by the genetic behavior of
top^{A4}, top^{R26} and top^{2W74}. top^{A4} encodes a lig^- kin^- ter^+ 
receptor, while top^{R26} and top^{2W74} encode lig^- kin^- ter^+ 
products; all three alleles, at room temperature, show
similar homozygous embryonic lethal phenotypes. How-
ever, top^{A4} generally shows more severe phenotypes in
combination with top^{E2}, top^{251} and top^{E1} than do
top^{R26} and top^{2W74} (Figure 4). In an analogous fashion,
the semiviable mutation top^{CA} (lig^- kin^- ter+ product)
partially complements top^{R26} and top^{2W74} but fails to
complement the pupal lethal allele top^{E8} (lig^- kin^- ter+ 
product) (Figure 4).

Alleles whose products have altered extracellular do-
main generally complement other mutant alleles less
completely than do equally (or, in some cases, more)
severe alleles encoding receptors with altered kinase or
carboxyl-terminal domains. These observations argue
that the extracellular domain of Torpedo is necessary
for interallelic complementation. Full complementa-
tion occurs between certain lig^- receptors and partial
complementation between lig^- and lig^- receptors.

Alternatively, the complementation behavior of the
class II mutations may reflect the increased cell surface
expression or stability of their protein products relative
to those encoded by other classes of torpedo alleles. If
they are expressed at higher levels than receptors en-
coded by the class I, III and IV alleles, the products of
the class II alleles would have a greater probability of
interacting with—thereby stabilizing or forming
functional heterodimers with—other mutant Torpedo
molecules.

Does signal transduction require ligand binding by
both receptors? top^{392} and top^{E1}, which encode pro-
teins with altered ligand-binding domains (Table 2), be-
have as total loss-of-function mutations when homozy-
gous or in trans to chromosomal deficiencies. However,
since these alleles partially complement torpedo muta-
tions encoding receptors with altered kinase domains
(Table 2, Figure 4), the Top^{392} and Top^{E1} proteins ap-
parently can form some functional heterodimers.

Missense mutations within the extracellular domain of
Torpedo could abolish receptor function, yet still al-
lo intermolecular complementation, in a number of
ways. One possibility is that the amino acid alterations
produced by top^{392} and top^{E1} only affect protein sta-
bility. If this were the case, complementation would re-
flect stabilization of the Top^{392} and Top^{E1} proteins
through the co-expression of another species of mutant
receptor.

Another possible mechanism for the inactivation of
Top^{392} and Top^{E1} is that they bind ligand and initiate
dimer formation normally, but do not form stable re-
ceptor complexes. The formation of short lived het-
erodimers between these molecules and kinase-
impaird Torpedo proteins might be sufficient for weak
signal transduction.

A third possibility is that these mutant Torpedo pro-
teins are defective in ligand binding and, consequently,
in the initiation of dimer formation. The stability of
dimers containing these proteins, however, might be
normal. Biochemical studies suggest that receptor
dimerization and signal transduction may require ligand
binding only by one member of the pair. Epidermal
growth factor stimulates the dimerization of EGF-R and
HER2 in vivo (Quan et al. 1992; Spivak-Kroizman et al.
1992), as well as the aggregation of purified EGF-R and
HER2 extracellular domains in solution (Spivak-
Kroizman et al. 1992). Neither HER2 nor its purified
extracellular domain possesses EGF binding activity.
When coexpressed with kinase^- EGF-R, HER2 protein
autophosphorylates and associates with PLC-g in re-
sponse to EGF stimulation (Spivak-Kroizman et al.
1992). In an analogous manner, the Top^{392} and Top^{E1}
proteins might be completely defective in ligand binding,
yet still be able to associate with and be activated by a
ligand-bound Torpedo molecule with a defective kinase
domain.

Conclusions: We have molecularly characterized 24
loss-of-function mutations at the torpedo locus of Dro-
sophila, which encodes a receptor tyrosine kinase of the
EGF receptor subfamily. Genetic interactions between
21 of these alleles were examined (this work; Clifford
and Schüpbach 1989). Based on the correlation ob-
served between the genetic behavior and amino acid
alterations of these mutations, we draw the following
conclusions: (i) Torpedo may be activated by tissue-
specific ligands, (ii) Torpedo may phosphorylate mul-
tiple substrates within the same tissue, (iii) signal trans-
duction by torpedo appears to require the physical
interaction of receptors, (iv) the extracellular domain of
Torpedo plays a critical role in mediating receptor-
receptor interactions. Our results complement and ex-
tend the previous work of Raz et al. (1991), and are
consistent with models of receptor tyrosine kinase func-
tion derived from tissue culture studies.

Using a functional (genetic) assay, we have identified
residues in the extracellular and intracellular domains of
Torpedo that are essential for signal transduction.
Since a number of the affected amino acids are con-
served between the the fly and vertebrate receptors, it
should be possible to investigate the biochemical con-
sequences of these mutant alterations on receptor func-
tion by engineering them into the human epidermal
growth factor receptor.
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LITERATURE CITED


Schubach, T., 1987 Germ line and soma cooperate during oogenesis to establish the dorsoventral pattern of egg shell and embryo.