Torso, a receptor tyrosine kinase required for embryonic pattern formation, shares substrates with the Sevenless and EGF-R pathways in Drosophila

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The maternally expressed Drosophila gene torso (tor) is a receptor tyrosine kinase that, when activated, initiates a signal transduction cascade that is responsible for the proper differentiation of the terminal, nonsegmented regions of the embryo. l[t]pole hole, the Drosophila raf-1 serine–threonine kinase homolog, and corkscrew, a tyrosine phosphatase, have been shown previously to function in this signal transduction pathway. We have identified other products in this pathway by carrying out a mutagenesis screen for dominant suppressors of a tor gain-of-function allele. More than 40 mutations, some of which fall into seven complementation groups, have been characterized genetically. Two of these correspond to mutations in ras-1 and Son of sevenless [Sos], which also function in the sevenless and EGF receptor [Der] tyrosine kinase pathways. The phenotypes of several other Su(tor) mutations suggest that they also function in other receptor tyrosine kinase-activated pathways at different times during Drosophila development.

[Key Words: Torso, Drosophila receptor tyrosine kinase; signal transduction; suppressors]

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During the development of most multicellular organisms, the determination of cell fate and subsequent differentiation require communication among cells, either over a distance or by direct cell–cell contact. Extracellular signals are often received and interpreted by signal transduction cascades that use a variety of proteins, such as kinases, phosphatases, and G proteins, many of which are functionally conserved over a wide range of species (Bourne et al. 1991; Greenwald and Rubin 1992; Pawson 1992). Given the complexity of signal transduction pathways, it is not surprising that certain mutations in pathway components are associated with cellular transformation and oncogenesis (Cantley et al. 1991), suggesting that these proteins are critical in the regulation of cell proliferation and/or differentiation.

A powerful tool for dissecting the different interactions in signaling pathways is to alter, by mutation, the activities of particular proteins. For these studies, systems in which genetics can be exploited have recently been very useful. In particular, the control of bud site selection in yeast, under the control of the BUD genes (Chant et al. 1991; Powers et al. 1991), and the induction of vulval cell fates during Caenorhabditis elegans development (Aroian et al. 1990; Beitel et al. 1990; Clark et al. 1992; Hill and Sternberg 1992, for review, see Greenwald and Rubin 1992; Sternberg and Horvitz 1992) are two pathways in which many members and their relationships are rapidly becoming better understood. In Drosophila, the signaling cascade regulated by the receptor tyrosine kinase, Sevenless (Sev), has also been studied extensively. Activation of Sev is required for the differentiation of one of the photoreceptor cells, R7, in the compound eye (for review, see Rubin 1991; Greenwald and Rubin 1992). Other components of the Sev pathway have been identified both by looking for mutants with common phenotypes and by screening for enhancers or suppressors of the sev mutant phenotype (Rogge et al. 1991; Simon et al. 1991; Gaul et al. 1992). Here, we have studied the signal transduction pathway regulated by torso, which encodes another Drosophila receptor tyrosine kinase that is required for embryonic development.

The initial specification of cell fate in the Drosophila embryo depends on four separate systems of maternally expressed genes. Three of these systems, anterior, posterior, and terminal, are required for the anteroposterior axis, and another is necessary for the dorsoventral axis (for review, see Nüsslein-Volhard et al. 1987; Stein and Stevens 1991; St. Johnston and Nüsslein-Volhard 1992). Each system includes one or more key gene products whose activity is localized and thereby confers the spatial information required for the differentiation of a specific region of the embryo. The anterior and posterior systems require the localization of the transcripts of the bicoid and nanos genes (Berleth et al. 1988; Wang and...
Lehmann 1991), respectively, and the local production and diffusion of the proteins that they encode provide the necessary spatially restricted activities that regulate downstream gene activities [Driever and Nusslein-Volhard 1988, Wang and Lehmann 1991]. In contrast, in the dorsoventral and terminal systems, no localized product has yet been found. The key genes in these two pattern-forming systems appear to be transmembrane receptors that are located around the entire circumference of the embryo but are only activated in a specific region, probably by spatially restricted ligands [Stevens et al. 1990; Stein et al. 1991]. As yet, the exact mechanism by which these receptors, Toll in the dorsoventral system and Torso in the terminal system, are activated is not understood, although other genes involved in the pathways have been identified [Stein and Stevens 1991; St. Johnston and Nusslein-Volhard 1992].

The terminal system is responsible for the specification of the nonsegmented termini of the embryo, called the acron and telson [Nüsslein-Volhard et al. 1987; Klingler et al. 1988]. The seven maternally active genes of the terminal class are: torso (tor), trunk (trk), fs(1)Nasrat ([fs(1)N]), fs(1)pole hole ([fs(1)ph]), torso-like ([tl]), l(1)pole hole ([l(1)ph]), and corkscrew (csw) [Perrimon et al. 1985; Klingler et al. 1988, Nishida et al. 1988; Schüpbach and Wieschaus 1989, Degelmann et al. 1990; Stevens et al. 1990; Perkins et al. 1992]. Loss-of-function [l-o-f] mutations in any one of these genes in the mother result in deletions of structures from both ends of the embryo. Specifically, in the anterior, the labrum is missing and the head skeleton is collapsed; in the posterior, all structures posterior to abdominal segment 7 are deleted, including abdominal segment 8, the anal plates, spiracles, filzkörper, and posterior midgut derivatives. Five of the terminal class genes appear to be strict maternal effect genes and are not required at other times in the Drosophila life cycle. The exceptions are l(1)ph and csw, which, in addition to their maternal function in the terminal pathway, are also required during larval stages for imaginal disc growth [Perrimon et al. 1985; Nishida et al. 1988, Perkins et al. 1992].

The three terminal class genes that have been cloned and characterized show significant homologies to other signaling proteins. The tor gene encodes an ~105-kD protein (Tor) containing a possible transmembrane segment and an intracellular domain with several features common to receptor tyrosine kinases [RTKs], with particular similarity to the kinase domain of the platelet-derived growth factor [PDGF] receptor [Casanova and Struhl 1989; Sprenger et al. 1989]. The tor transcript is detectable throughout the early embryo [Sprenger et al. 1989] and antibodies against Tor show that it is associated with the early embryonic membrane in a uniform distribution around the entire circumference of the embryo [Casanova and Struhl 1989]. The l(1)ph gene encodes the Drosophila Raf-1 homolog, a serine–threonine kinase [Mark et al. 1987; Nishida et al. 1988]. It has been reported recently that csw encodes a non-receptor tyrosine phosphatase that is homologous to the mammalian PTP1C protein [Perkins et al. 1992]. The maternally transcribed l(1)ph and csw transcripts are distributed throughout the mature oocyte and early embryo [Nishida et al. 1988; Ambrosio et al. 1989a, Perkins et al. 1992].

Of the terminal class genes, tor is unique in having gain-of-function [g-o-f] alleles that show a phenotype opposite that shown by the l-o-f alleles. The phenotype of embryos derived from tor g-o-f mutant mothers [hereafter referred to as mutant embryos] is an apparent absence of segmentation in the middle body region and an expansion of terminal structures [Klingler et al. 1988; Schüpbach and Wieschaus 1989]. These tor g-o-f alleles have been used to show that four of the other terminal class gene products act upstream of Tor. Double mutants with tor g-o-f mutations and trk, tsk, fs(1)N, or fs(1)ph l-o-f mutations still show the tor g-o-f phenotype, implying that Tor activity is independent of those terminal gene functions [Klingler et al. 1988; Stevens et al. 1990]. The exceptions are [l]ph and csw, which are required for the expression of the tor g-o-f phenotypes and therefore appear to function downstream of Tor [Ambrosio et al. 1989b, Klingler 1989; Perkins et al. 1992]. In addition to these maternal genes, two zygotic gap genes, tailless [tl] and huckebein [hb], are transcribed in domains restricted to the poles of the embryo, and have mutant phenotypes similar to the maternal terminal class genes [Pignoni et al. 1990; Brönnner and Jäckle 1991].

It is probable that additional maternal products are required in the signal transduction pathway activated by Tor. Like l(1)ph and csw, these components may not have been identified in previous screens, because they are required at other times in development and are consequently lethal when mutated. In an effort to identify other genes in the terminal pathway, we undertook a large-scale mutagenesis screen for mutations that act as dominant suppressors of the tor g-o-f allele, torRL3. We chose this recessive allele for such a screen because it is temperature sensitive, suggesting that its phenotype might be suppressible by a partial reduction of the amount of a product required in the pathway. We identified 45 mutations that suppress the torRL3 phenotype when heterozygous, 17 of these fall into seven complementation groups of more than one member. By complementation tests with mutations identified as enhancers of a hypomorphic allele of sev [E(sev)] loci [Simon et al. 1991] we show that four of these mutations disrupt the Son of sevenless [Sos] or ras-I loci. We also show that, in addition to ras-I and Sos, two other E(sev) mutations suppress the torRL3 phenotype, indicating that the Tor and Sev pathways overlap extensively. Of the new Su(r) mutations that we isolated, most are lethal mutations, suggesting that they may have other critical roles besides that in the Tor pathway. In addition, several Su(r) loci display maternal or adult mutant phenotypes suggesting that they also act in the epidermal growth factor receptor [EGF-R or Der] pathway at different times in the life cycle. Thus, the suppressor of the tor g-o-f genetic screen that we describe here has proved to be a powerful method to identify genes that participate in a variety of signaling pathways.
Results

Temperature sensitivity of torRL3

Because products that function downstream of Tor are likely to act in other signaling pathways, mutations in these genes may not be identified in screens for strict maternal effect mutations. For example, if these gene products are involved in cell proliferation and/or differentiation, as l(1)ph and csw are, mutations in them are likely to be lethal during embryonic or larval stages. Therefore, we designed a screen to isolate signal transduction mutations in heterozygous mothers. By looking for mutations that act as dominant suppressors of the tor g-o-f phenotype, it may be possible to identify these mutations in the heterozygous condition. Although most genes are not haploinsufficient when studied in a wildtype background, it is possible to manipulate the conditions to make the function of some genes dosage dependent. In this case, we chose to carry out the screen at a temperature at which the torRL3 phenotype might be sensitive to approximately twofold changes in the levels of potential downstream substrates. The heterozygous mutations were not expected to have an effect on the development or viability of the flies or on the fertility of females that are wild type for tor.

The temperature sensitivity of torRL3 was advantageous in several ways. First, the torRL3 allele is sensitive to genetic background as well as to temperature changes of a few degrees, and it seemed likely that its phenotype would also be sensitive to changes in the amounts of substrates. Second, because homozygous torRL3 females show 100% sterility at 27°C, individual females carrying one copy of a strong suppressor mutation could be detected by even a small fraction of hatching embryos. Third, homozygous torRL3 mothers are fertile at 18°C, so progeny containing a suppressor mutation were recovered by shifting the mother to 18°C. This feature as well as the viability and fertility of torRL3 homozygous males enabled the screen to be done as a maternal effect screen in the F1 generation [see Fig. 2, below]. It should be noted that in addition to l-o-f mutations in haploinsufficient genes, this type of screen might also detect neomorphic g-o-f and dominant-negative mutations.

Figure 1 shows a comparison of the cuticle phenotype of wild type (a), tor l-o-f (b), and g-o-f torRL3 mutants at 22°C and 27°C (c and d, respectively). The tor l-o-f mutant embryo exhibits defects at both the anterior and posterior poles. In contrast, in torRL3 embryos, terminal structures are present, but there is a disruption of segmentation in the middle region of the embryo [Klingler 1989; Schüpbach and Wieschaus 1989]. Although the penetrance of the torRL3 mutation is 100% at 27°C [i.e., all embryos show some mutant phenotype], the strength of the phenotype can vary even among embryos from a single female. Of eggs collected from females at 27°C, <10% had one or more ventral denticle belts, 5–10% had extra rudimentary filzkörper material and/or a duplicated tuft, and <1% hatched into first-instar larvae (Fig. 1d). Virtually all embryos produced some cuticle material, but they were extremely disorganized in comparison with embryos collected at lower temperatures. At 22°C, a significantly higher percentage of embryos hatched (40–50%), and all embryos showed a more organized anteroposterior axis, including at least several denticle belts (Fig. 1c). Thus, 5°C changes in the temperature of egg collections show a gradual but reproducible change in the mutant phenotype.

Isolation of suppressors of torRL3 [Su(tor) loci]

The basic strategy of the screen is shown in Figure 2 and described in more detail in Materials and methods. Fifteen thousand individual females were screened, ~160 produced embryos with a suppressed phenotype, and progeny were recovered from 130 of these. In the secondary screen, the criteria for suppression were more stringent, both with respect to penetrance and expressivity, and several more lines were eliminated because they failed to show a highly penetrant phenotype. The scheme for the secondary screen and linkage analysis is shown in Figure 2b, and the results are summarized in Table 1. Once stocks were established, the Su(tor) loci were rescreeened more carefully by cuticle preparations, and at this point only 45 individual lines were maintained. The final number of recovered suppressors was 6 mutations on the X chromosome, 26 mutations on the second chromosome, and 13 mutations on the third chromosome.

Phenotype of suppressed embryos

Because the suppression screen did not require that embryos survive to adulthood, Su(tor) mutations with a wide range of effects were isolated. Examples of two different classes of Su(tor) effects are shown in Figure 3. Females carrying a weak Su(tor) mutation gave rise to no hatching larva, and all the embryos showed a weak but consistently suppressed phenotype (Fig. 3b) when compared with torRL3 (Fig. 3a). In contrast, strong Su(tor) mutations produced embryos with a highly repressed phenotype [Fig. 3c] and 10–20% hatching larva, although these exhibited weak segmentation defects characteristic of torRL3 at 22°C and generally did not survive. In addition, the penetrance of some of the Su(tor) mutations was somewhat variable.

To assess how early the Su(tor) mutations are acting, we examined the expression of till, which is likely to be one of the first transcriptional targets of the Tor signal transduction pathway. Figure 4 compares the till mRNA expression in a wild-type embryo [Fig. 4a], in an unsuppressed torRL3 embryo at 27°C [Fig. 4b], and in a torRL3 embryo carrying a suppressor [Fig. 4c; Su(tor)2-48]. In torRL3 mutants, the till expression pattern is expanded and variable, particularly in the anterior of the embryo. In the posterior, the till expression domain extends anteriorly over one-third to one-half the embryo. In the suppressed embryo, till expression looks almost wild type, with only a slight expansion of the posterior domain.

Complementation tests

For complementation tests of dominant Su(tor) loci, sev-
Figure 1. The temperature sensitive g-o-f \textit{tor} \textit{RL3} allele exhibits a phenotype that is opposite that of a l-o-f \textit{tor} allele. Cuticle preparations were done of unhatched larvae to compare the phenotypes of wild-type and various \textit{tor} mutants to illustrate the temperature sensitivity of \textit{tor} \textit{RL3}. Embryos are oriented anterior up, ventral to the left, lateral views are shown. In a wild-type embryo (a), \textit{tor} function is required for the development of the terminal regions of the embryo, including the labrum, a part of the head skeleton, and structures posterior to abdominal segment 7 (A7), such as the anal plates and posterior spiracles (arrows). (b) In a \textit{tor} l-o-f mutant embryo (\textit{tor} \textit{VM}/ \textit{tor} \textit{VM}), terminal structures are missing: The head skeleton is collapsed, and A7 is seen at the posterior-most point of the embryo. The g-o-f \textit{tor} \textit{RL3} allele is temperature sensitive and shows defects in the abdominal region of the embryo but not at the termini. (c) At 22°C, embryos display between five and eight abdominal denticle belts and have normal head and telson structures, including well extended filzkörper (arrow). Segmentation disruptions are primarily seen in the A2 to A5 region. (d) At 27°C, most abdominal segments are deleted, and embryos differentiate only terminal structures, including spiracles, filzkörper material (arrow), anal plates, and tuft (not visible in this focal plane), and often display disorganized head skeletons.

Several different aspects of their phenotypes were assayed. First, mutations were tested in a straightforward lethal complementation assay. Those flies that did not show a lethal interaction were examined for an obvious adult phenotype. Finally, viable females that carried two separately isolated \textit{Su(tor)} mutations were placed into egg-laying blocks and their progeny examined for a phenotype resulting from a maternal effect. The results of these assays are described below and summarized in Table 1.

By analogy to \textit{l(1)ph} and \textit{csw}, we suspected that the \textit{Su(tor)} mutations would act maternally to suppress \textit{tor} \textit{RL3} but might also be zygotic lethal. We tested whether we had obtained any lethal complementation groups by crossing balanced suppressor stocks with each other. For the second chromosome crosses, we found three lethal complementation groups, one with three members (2-1) and the others with two each (2-2 and 2-3). Two lethal groups (3-1 and 3-2) with two members each were found on the third chromosome. None of the other crosses showed 100% lethality.

There were two viable second chromosome \textit{Su(tor)} mutations [\textit{Su(tor)}2-68 and \textit{Su(tor)}2-417] that had slightly rough eyes as homozygotes. When these mutations were crossed with each other, the transheterozygous flies again had rough eyes. This suggests that these two mutations might be affecting other RTK pathways, such as the Sev or DER pathway, that are involved in eye patterning. When one of these mutations, \textit{Su(tor)}2-417, was crossed with 20 other second chromosome suppressors, no visible adult phenotypes were seen. The specificity of the rough eye phenotype seen with \textit{Su(tor)}2-417 and \textit{Su(tor)}2-68 transheterozygotes suggests that these mutations are allelic.

The embryos of viable transheterozygous females were examined for a maternal effect. Our expectation was that a homozygous suppressor that was a l-o-f mutation
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most of these were scored in the presence of tor<sup>Rl3</sup>, they showed variable degrees of suppression at 27°C, and a high percentage of hatching embryos at 18°C, as expected. However, in a few cases, trans-heterozygous females produced embryos that did not hatch and, on closer examination, were seen to have chorion defects. These defects probably result from a disruption of the DER pathway required for follicle cell and embryonic patterning (see below and Fig. 6). This maternal phenotype was not highly penetrant and was seen primarily with several combinations of second chromosome-linked Su(tor) mutations, as indicated in Table 1.

Four viable X-linked Su(tor) mutations were female sterile as homozygotes and produced eggs that were discolored and collapsed. When these mutations were crossed together, the same female sterile phenotype was obtained. These mutations are allelic and appear to define a viable female sterile complementation group, indicated as Su(tor)1-1 in Table 1.

It should be noted that it is possible that mutations complement each other in the above assays but actually disrupt the same locus, as they were not isolated because they showed a phenotype on their own. That is, we do not know what phenotype a homozygous mutant will show. Such complementation can be resolved by mapping or possibly by the isolation of more alleles. Conversely, we have also detected nonallelic lethality between Su(tor)2-85 and Su(tor)2-93, which map to different arms of the second chromosome. Both of these are unhealthy stocks and show partial dominant female sterility, suggesting that they are not straightforward 1-o-f mutations.

Maternal vs. zygotic requirement

Because the gap segmentation genes are among the earliest genes transcribed from the zygotic genome, we predicted that the components of the Tor signal transduction pathway required for tll and hkb expression would be provided maternally. We therefore classified further the Su(tor) mutations by testing whether they function maternally or zygotically. This was done by crossing males that were heterozygous for the suppressors to homozygous tor<sup>Rl3</sup> females and scoring the cuticle phenotype of the progeny. Half of the progeny should inherit the suppressor from their father. If the suppressor is required maternally, the genotype of the father has no influence, and no suppression should be seen. Table 1 shows the classification into maternally and zygotically active groups. There are 38 maternally active and 7 zygotically active Su(tor) mutations. In addition, there are a few cases in which a Su(tor) mutation seems to be active both maternally and zygotically. It is possible that a mutation could be a zygotic suppressor, even if its wild-type product is normally needed maternally, for example, by acting as a dominant-negative mutation that interferes with the wild-type, maternally provided product.

Mapping of Su(tor) mutations

The maternal-effect suppressors that showed the most
Table 1. Summary of Su(tor) mutations

| Stock name* | v or l | m or z | Group | Position | Elp | Comments |
|-------------|--------|--------|--------|----------|-----|----------|
| First chromosome | | | | | | |
| 1-19 | v (FS) | m | 1-1 | cv and c | weak | homozygous females |
| 1-134 | v (FS) | m | 1-1 | cv and c | weak | produce collapsed eggs |
| 1-285 | v (FS) | m | 1-1 | cv and c | | |
| 1-352 | v (FS) | m | 1-1 | cv and c | | |
| 1-106 | v | | | | | |
| 1-163 | v | m | | | no | |
| Second chromosome | | | | | | |
| 2-15 | l | z | 2-1 | ~2-16 | no | |
| 2-17 | l | m | | ~2-60 | no | |
| 2-28 | l | m | | near cn | no | |
| 2-48 | l | m | | | | |
| 2-56 | v | m | 2-1 | ~2-16 | no | |
| 2-60 | l | m | 2-4 | | no | rough eyes |
| 2-68 | v | m | | | yes* | chorion interactions |
| 2-83 | l | m | 2-3 | | yes* | |
| 2-85 | l | m | 2-1 | | no | semidominant FS |
| 2-87 | v | m | | | | |
| 2-93 | l | m | | ~2-76 | yes* | chorion interactions |
| 2-133 | v | m | 2-2 Sos | 34D | yes* | |
| 2-157 | l | m | 2-60 | no | | |
| 2-180 | l | m | | c and bw | yes* | semidominant FS |
| 2-187-1 | l | m | | | no | |
| 2-187-3 | v | m | | | | |
| 2-191 | v | m | | | | |
| 2-237 | l | m, | | ~2-95 | yes | |
| 2-278 | l | m, z | | weak | | |
| 2-280 | v | m | | | | |
| 2-321 | l | m | | weak | | |
| 2-341 | l | m | | | | |
| 2-361 | l | m | 2-3 | | ~2-60 | weak |
| 2-399 | v | z | | | | |
| 2-405 | l | m | 2-2 Sos | 34D | yes | |
| 2-408 | l | m | | weak | | |
| 2-417 | v | m | 2-4 | | yes | rough eyes |
| Third chromosome | | | | | | |
| 3-8 | l | m, z | 3-1 | | ~3-48 | weak* |
| 3-38 | l | m | 3-1 | | ~3-48 | |
| 3-42 | l | m | 3-1 | | | |
| 3-91 | l | z | | | | |
| 3-168 | v | z | | | | |
| 3-293 | l | z | | | | |
| 3-307 | l | m | | | | |
| 3-333 | v | z | | | | |
| 3-337 | l | z | | | | |
| 3-341 | l | m, | 3-2 tas-1 | 85D | weak | |
| 3-344 | v | m | | | | |
| 3-404 | l | m, z | 3-2 tas-1 | 85D | yes | |

The Su(tor) mutations define seven complementation groups and exhibit a variety of phenotypes. Many of the characteristics of the Su(tor) mutations that were assayed as described in Results and Materials and methods are summarized here.

*Chromosomal linkage and stock numbers.

*The viability [v] or lethality [l] of the Su(tor) chromosome as a homozygote is indicated. [FS] Female sterility. [Note that in most cases the Su(tor) mutation has not been recombined off the mutagenized chromosome, so the observed lethality may be caused by a second mutation on the chromosome.]

*Maternal [m] or zygotic [z] action of the suppressor.

*Complementation group and identity.

*Map positions.

*Su(tor) stocks that were tested for suppression of Elp and the results of those assays [those with an asterisk were scored using SEM (see Fig. 6)].
Ras-1 and Sos are in the Torso pathway.

map position and to choose recessive visible markers for further mapping. In some cases we were also able to test whether recombinant chromosomes still carried both the suppressor and a lethal mutation. More detailed recombination mapping was done for a few of the Su[tor] loci (see Materials and methods). The results of the mapping are shown in Table 1.

Complementation with E(sev) mutations

One of the lethal complementation groups, Su[tor]2-2,
mapped very close to Sos [Rogge et al. 1991; Simon et al. 1991]. We obtained 1-o-f lethal Sos alleles and found that our two Su[tor]2-2 mutations failed to complement them, whereas 10 other second chromosome-linked Su[tor] loci did complement Sos. It has been shown that Sos encodes a protein with putative homology to the Saccharomyces cerevisiae cdc 25 gene product, a guanine nucleotide exchange factor that is proposed to be involved in the Ras pathway. This suggested that substrates in the Sev pathway may also be used in the Tor pathway. We therefore carried out complementation tests with the other E[sev] mutations. From these tests we found that the lethal pair, Su[tor]3-341 and Su[tor]3-404, failed to complement the lethality of E[sev]3C[eb] and E[sev]3C[aw], which are mutations in the ras-1 gene. Thus, both ras-1 and Sos function in both the Tor and Sev pathways, indicating that the screen was successful in isolating mutations in known signaling genes. The phenotype of tor[R13] embryos carrying the ras-1 and Sos mutations is shown in Figure 5, a and b, respectively. The other crosses between E[sev] and Su[tor] mutations were viable and did not show a visible adult phenotype, and trans-heterozygous females showed no maternal effect on the chorion structure or embryonic pattern formation.

From the results described above, it appears that RTK-activated pathways share at least some substrates. We therefore tested the other E[sev] mutations for an ability to suppress tor[R13]. The E[sev] mutations were isolated in a screen for enhancers of a hypomorphic sev allele, that is, for mutations that reduce sev activity [Simon et al. 1991]. By testing for their ability to suppress the g-o-f tor[R13] allele, we are also looking for a reduction of Tor activity. The E[sev]2B mutation, represented by a single allele, was recombined onto a tor[R13] chromosome and backcrossed to tor[R13]. Females homozygous for tor[R13] and heterozygous for E[sev]2B produced suppressed embryos when compared with control embryos lacking the E[sev]2B mutation [Fig. 5c]. The E[sev]2B mutation appears to be a weak suppressor compared with ras-1 and Sos mutations (Fig. 5c, cf. c with a and b).

The other three E[sev] loci linked to the third chromosome were crossed into a homozygous tor[R13] stock, and embryos were scored for suppression. The two E[sev]3A alleles showed weak suppression, whereas E[sev]3B and E[sev]3D did not show convincing suppression over several days of analysis. The two E[sev]1A alleles on the X chromosome show very strong, highly penetrant suppression of tor[R13] (Fig. 5d). On the basis of their map position and failure to complement, these E[sev]1A mutations are likely to be alleles of csw [Simon et al. 1991; Perkins et al. 1992]. These same E[sev] mutations have been shown to suppress Elp, a dominant allele of the EGF-R [Der], indicating that the Sev, Der, and Tor RTK pathways contain common signaling components.

Interactions in the Drosophila EGF-R (Der) pathway

As mentioned previously, weak interactions between some Su[tor] loci suggested a disruption of the Der sig-

![Figure 5](https://example.com/figure5.png)

Figure 5. Several E[sev] mutations including ras-1 and Sos also act as suppressors of tor[R13]. Cuticle preparations were done on late-staged tor[R13] embryos containing a Su[tor] or E[sev] mutation, at 27°C. All embryos are oriented with anterior to the left, ventral down, and lateral views are shown. Two of the lethal Su[tor] complementation groups were found to be allelic to mutations in ras-1 and Sos. Both ras-1 [a] and Sos [b] act as strong, highly penetrant suppressors, giving rise to embryos with four to six abdominal denticle belts, as seen in tor[R13] embryos at 22°C (cf. Fig. 1c). Both also show normal termini. [c] E[sev]2B or crk-like, a putative SH2-containing adaptor molecule, is a fairly weak suppressor. These embryos generally are more disorganized than those shown in a and b and cannot be easily removed from their vitelline membrane. [d] E[sev]1A appears to correspond to a csw mutation and also acts as a suppressor of tor[R13]. These suppressed embryos are intermediate in phenotype and are usually weaker than ras-1 [a] or Sos [b] but stronger than E[sev]2B [c].
transheterozygotes, several Su(tor) mutations show partial female sterility and produce embryos with obvious chorion defects, two examples of which are shown in Figure 6, b and c. These phenotypes can be interpreted as a partial ventralization of the chorion pattern. A similar phenotype is produced by several other maternal-effect mutations, including the torpedo (top) alleles of Der (Schüpbach and Wieschaus 1989). The Su(tor) combinations in which this phenotype is seen are indicated in Table 1.

We also tested several of the Su(tor) mutations for their ability to suppress the Elp rough eye phenotype. Elp appears to encode a hyperactive Der protein, resulting in disorganized ommatidia and a rough eye (Baker and Rubin 1989). Adult flies that were heterozygous for Elp and for a single Su(tor) mutation were scored for a suppression of the rough eye, and several samples were chosen for scanning electron microscopy (SEM). An example of a wild-type eye, an Elp eye, and a Su(tor), Elp eye are shown in Figure 7, a–c. The Su(tor) mutations that were tested in this assay are indicated in Table 1.

Discussion

We have used a temperature-sensitive g-o-f allele of the maternal terminal-class gene tor to identify components of the signal transduction pathway that is activated by the receptor tyrosine kinase Tor. In a large-scale screen for suppressors of the tor g-o-f phenotype, we isolated mutations in five lethal and two viable complementation groups, as well as >20 apparently “single hit” mutations. The Su(tor) mutations are dominant because females carrying one copy of a given mutation show suppression of the torRL3 homozygous phenotype. Suppression can be characterized by a restoration of abdominal segments and a generally more organized cuticle and also by a correlative normalization of the tll expression pattern. These Su(tor) mutations could be acting in several ways, for example, as 1-o-f mutations that reduce the amount of a product in the pathway (i.e., a dosage effect), as dominant negatives, or as overactive negative regulators of the pathway. On the basis of the low allele frequency and interactions between apparently nonallelic loci, it is likely that many of the Su(tor) mutations are not simple 1-o-f alleles. They could, for example, be g-o-f mutations that act as hypermorphs or dominant negatives. While this type of dominant screen is clearly productive, not all signaling genes can be identified in this way. For example, we assayed previously isolated 1-o-f alleles of itlph and tll, and neither was able to suppress torRL3 under the conditions of our screen (data not shown). Previous reports, however, suggested that itlph and tll mutations showed a dosage effect on the viability of torRL3 embryos (Ambrosio et al. 1989b; Strecker et al. 1989). The difference between these results may be explained by the different assay conditions, such as temperature and genetic background.

Homology with other signaling proteins

Screens for suppressors or enhancers of the RTK, Sev,
Figure 7. The Su(tor) mutations can function in the Der pathway to suppress the Elp phenotype. Several Su(tor) mutations were crossed with Elp, a dominant hyperactive allele of Der that results in a rough eye phenotype, and eyes of transheterozygous Elp/Su(tor) flies were examined under a low-power microscope. Approximately half of the crosses showed a consistent suppression of Elp, as indicated in Table 1. Several samples were examined in more detail using SEM. Eyes are oriented anterior to the left, and dorsal up. (a) The wild-type eye shows a regular array of hexagonal ommatidia with alternately spaced hairs. (b) The Elp eye is smaller and rougher around the perimeter. The ommatidia are not arranged properly, and the hairs emerge in an irregular pattern. (c) An Elp/Su(tor)2-93 eye displays a more organized array of ommatidia compared with b, and most of the hairs are also regularly spaced. Magnification, 200× for a and c; 220× for b.
doubt be able to assign more roles to RTKs and their downstream effectors.

Overlap in the pathways

Several of the downstream effectors of Tor, Sev, and Der described above appear to be generally expressed and to act in multiple pathways. The sophisticated genetics of Drosophila has allowed us to identify these signaling molecules by focusing on a particular in vivo function while not disrupting others. Because of this extensive overlap, it may be difficult to understand the various mutant phenotypes and to dissect the functional relationships between signaling molecules. Because most of the Su(tor) mutations are autosomal linked and lethal, their maternal-effect phenotype cannot be looked at directly by inducing germ line clones by mitotic recombination. Another way to test for a maternal-effect, germ-line-dependent phenotype is to transplant potentially mutant pole cells into a sterile ovoD host (Lehmann and Nüsslein-Volhard 1986). However, it is likely that some of these signaling genes may be required earlier in oogenesis, for example, for germ cell proliferation; therefore, it is possible that homozygous mutant germ-line clones could not be recovered. For example, in addition to its role in the Der and Tor pathways, Ras-1 activity may also be required early in oogenesis for the proliferation and/or maturation of germ cells (C. Berg, pers. comm.). It is likely that ras-1 homozygous mutant germ-line clones generated by pole cell transplantations or by mitotic recombination would not be recovered.

Moreover, it may be difficult to look at the maternal homozygous mutant phenotype because of influences from the surrounding follicle cells. Although Tor and Der are required in different tissues of the female, they appear to activate signal transduction cascades that contain many common elements. For example, gap-1 mutations affect dorsoventral patterning in the follicular epithelium during oogenesis, which leads to the differentiation of dorsIALIZED embryos (Gaul et al. 1992; H. Ruohola-Baker, E. Grell, D. Baker, L.Y. Jan, and Y.N. Jan, in prep.). This phenotype appears to result from the overactivity of the Der signaling pathway in the follicle cells, which is transmitted to the embryo (Schniebacher 1987). Preliminary experiments indicate that a gap-1 mutation can enhance the torRL3 phenotype, suggesting that Gap-1 functions as a negative regulator in the Tor pathway (H. Doyle, unpub.), as it does in the Sev and Der pathways (Gaul et al. 1992; H. Ruohola-Baker, E. Grell, D. Baker, L.Y. Jan, and Y.N. Jan, in prep.). In addition, several of our Su(tor) mutations partially affect the pattern of the chorion, implying that they too might act downstream of Der. Thus, the Der and Tor pathways show extensive overlap, though the ultimate targets are probably different. These maternal systems provide an elegant example of the economical use of signaling molecules: The cascades are activated in adjacent tissues, in the follicular epithelium during oogenesis, and in the egg after fertilization, by the stimulation of RTKs by ligands presumably originating in part from the other tissue.

Model of the terminal system

A summary of the terminal system signaling cascade is shown in Figure 8. Previous work has shown that trk, fs(1)ph, fs(1)N, and tsl act upstream of tor (Klingler 1989; Stevens et al. 1990). It is not known yet whether any of these genes encodes the ligand or whether any or all are involved in the spatial regulation of ligand production. Because some fs(1)ph and fs(1)N alleles also give rise to unfertilized, collapsed eggs, their effects on the Tor pathway may be indirect (Degelmann et al. 1990). Of these maternal terminal-class genes, tsl is the only one that is required in the follicle cells, specifically in those cells located at the anterior and posterior ends of the egg chamber (Stevens et al. 1990). Thus, tsl may play a key role in generating asymmetry in the terminal system.

Previous results and this study indicate that five known signaling proteins with homology to vertebrate proteins act downstream of Tor. Based loosely on analogy to other systems (Clark et al. 1992; Lowenstein et al. 1992; Wood et al. 1992), we have arranged these products in a signaling pathway, but it should be emphasized that there is no data yet addressing direct interactions between these proteins in the Tor pathway. Because the double mutant phenotype is stronger than either mutant alone, Perkins et al. (1992) suggested that Csw and Raf-1 act in parallel, rather than in a strictly linear pathway. Our preliminary results suggest that the pathway is not simply a cascade of positive interactions and that there are negative inputs, as gap-1 mutations and deficiencies of some regions appear to enhance the activity of the torRL3 pathway (H. Doyle, unpub.).

Figure 8. The Tor signal transduction pathway includes at least five known signaling molecules. It was shown previously that [I(1)ph], the Raf-1 homolog, and csw, a putative tyrosine phosphatase, are required in this pathway. Our results implicate the activity of three other recently identified signaling genes—ras-1, Sos, and crk-le—which were first identified in the Sev pathway. The activities of these proteins are presented here in a model based partly on results from other systems, though we have not yet determined the epistatic relationships between these proteins in the Tor pathway. The end result of Tor activation is the transcription of the gap genes till and hkb in domains that are restricted to the poles of the embryo that give rise to terminal structures of the larvae. (For details and references, see discussion.)
It is probable that the earliest transcriptional targets of the Tor pathway are the zygotic gap genes $tll$ and $hkb$ (Strecker et al. 1989; Pignoni et al. 1990; Brömmner and Jäckle 1991; Steingrimsson et al. 1991). Spatially restricted expression of these genes is detected several nuclear cycles before cellular blastoderm formation, around the time of other gap gene expression. The transcription factors that regulate $tll$ and $hkb$ expression are probably already present in the freshly fertilized egg and are activated, perhaps by phosphorylation, as the final step of Tor activation. Some of the other $Su(tor)$ genes identified in this screen may correspond to these proposed factors, as well as to other components of the Tor signaling pathway. We are currently pursuing a more extensive genetic and molecular investigation of several of these genes. Several of the apparently single-hit alleles are of particular interest because of their possible g-of or neomorphic effects.

We have shown that the Tor RTK pathway contains many components with homology to vertebrate growth control genes and oncogenes. Many of these important signaling components are recruited into signal transduction pathways in a variety of tissues throughout the development of the fly, including pathways activated by the RTKs Sev and Der. Thus far, it has not been determined where the specificity in these pathways lies, such that at the appropriate time and place, terminal larval structures or specific photoreceptors will differentiate in a reproducible manner. Further studies of these signaling mutations and the proteins they encode, as well as the identification of other components, will help elucidate the details of these critical signal transduction pathways.

**Materials and Methods**

**Stocks**

The markers and balancer chromosomes used are described in *The Genome of Drosophila melanogaster* (Lindsley and Zimm 1992). The $tor^{R13}$ (also called *spliced*) and $tor^{SM}$ alleles were obtained from Trudi Schüpbach (Schüpbach and Wieschaus 1989).

Approximately 200 homozygous $b$ pr $tor^{R13}$ cn males were treated with 35 mM ethylmethanesulfonate (EMS) in 1% sucrose for 24 hr (Lewis and Bacher 1968). They were allowed to recover on standard fly media for 12 hr before being mated with ~1000 $tor^{R13}$ cn bw homozygous females (see Fig. 2). The flies were allowed to mate and lay eggs at 18°C. The parents were transferred to fresh bottles every 24 hr, and each bottle was kept at 18°C for 24 hr after the parents were removed, to inhibit any g-of activity of $tor^{R13}$, and then moved to 25°C until eclosion. All female progeny were collected as virgins, mated en masse to suitably marked males, and, after 1–2 days, placed into individual egg-laying blocks. We chose to do the screen at 27°C to eliminate background hatching and because the unsuppressed $tor^{R13}$ mutant phenotype is very consistent at this temperature. The eggs were collected for 24 hr at 27°C, and their phenotype was scored through the chorion under oil over a 3-day period (the first collection was not scored to allow time for temperature equilibration). Embryo collections in which >50% of the embryos showed two or more abdominal denticle belts were considered to be suppressed. Females that produced embryos showing a suppressed phenotype in two 24-hr collections were removed from the blocks and placed in fresh vials at 18°C. Progeny were recovered, and individual balanced lines were established. A secondary screen was done to determine the chromosomal linkage of the putative $Su(tor)$ loci and to eliminate non-reproducible effects. Fifteen thousand individual females were screened, and 160 suppressors were identified in the primary screen. Of these, 130 gave viable progeny. Individual stocks were established by the strategy outlined in Figure 2. They were screened once again using cuticle preparations and then reduced to 45 lines.

The $tor^{R13}$ mutation is closely linked (<0.5% recombination) to the visible eye color gene, $cn$. In all subsequent crosses we could follow the $tor^{R13}$ gene by looking for $cn$. This was important because we needed to re-screen and map the $Su(tor)$ mutations in the presence of $tor^{R13}$.

**Recombination mapping**

Several of the strong $Su(tor)$ mutations located on the second chromosome were initially mapped very roughly using a second chromosome containing the dominant markers with $S$ Sp Bl Tfr L. Individual recombinant females that were homozygous for $tor^{R13}$ (i.e., $cn/cn$) were assayed for suppressed embryos. In this way, a rough map position relative to $tor$ was obtained. In most cases, several recombinant chromosomes were backcrossed to the original $Su(tor)$ stock to see whether the suppressor could be separated from the lethality. Those stocks in which the suppressor and the lethal seemed to be closely linked were mapped more accurately as lethals, independent of their affect on $tor^{R13}$. The stocks with more than one lethal allele were mapped first as suppressors and then as lethals using the independent alleles. For these finer mapping studies, the multiply marked second chromosome $al$ $dp$ $pr$ $c$ $px$ $sp$ was used with the tester chromosome $al$ $dp$ $pr$ $Bl$ $c$ $px$ $sp$. The map positions of the mutations for which a large number of recombinants were obtained are given in approximate recombination units in Table 1, whereas those for which fewer recombinants were obtained are shown only relative to visible markers.

The $Su(tor)$ mutations linked to the third chromosome were more difficult to map as suppressors in a homozygous $tor^{R13}$ background. We therefore only attempted to map the two lethal complementation groups, $Su(tor)3-1$ and $Su(tor)3-2$, making the assumption that the lethality and suppressor mapped together. Recombinant lethal chromosomes that were retested for their ability to suppress $tor^{R13}$ confirmed this assumption. The $Su(tor)3-2$ mutations were found to be alleles of $ras-1$, so the recombination mapping was not continued. We initially mapped the lethality of $Su(tor)3-1$ between st and ct, using the chromosome marked $ru$ $b$ st $st$ $cu$ $sr$ $e^e$ $ca$. We then mapped these mutations using the $mvh$ $th$ $st$ $ri$ $roe$ $p^e$ $cu$ $sr$ $e^e$ chromosome to lie at ~3–48. Because of the difficulty in mapping the third chromosome-linked mutations in a homozygous $tor^{R13}$ background, the other mutations were not mapped.

As indicated in Table 1, one female sterile complementation group, $Su(tor)1-1$, was linked to the X chromosome. Homozygous females carrying any combination of these four alleles produce eggs with discolored and collapsed chorions. We used this female sterile phenotype to map this mutation to a position roughly between $cv$ and $ct$, at approximately map position 18–19.

**Cuticle and chorion preparations**

Cuticle preparations were done in Hoyers medium as described (Wieschaus and Nüsslein-Volhard 1986) and examined using dark-field microscopy. The vitelline membranes were removed...
either by pressing on the coverslip or by shaking in a mixture of heptane and methanol before mounting in Hoyers. Embryos displaying a strong tor mutant phenotype collapse when the vitelline membrane is removed, so this membrane was left intact in some preparations. Eggs with chorions were gently rinsed from egg collection plates and mounted directly in Hoyers without fixation.

**Whole-mount in situ hybridization**

The il2 cDNA clone was obtained from Pignoni et al. (1990), and the insert was labeled with digoxigenin-11-UTP by random priming (Boehringer-Mannheim). Embryos were prepared for whole-mount in situ hybridization as described previously (Tautz and Pfeifle 1989), and the signal was detected using the Genius Kit (Boehringer-Mannheim).

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**Note added in proof**

The name of the gene crk-le has apparently been changed to drk, or downstream of regulated kinase (G.M. Rubin, pers. comm.).

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Torso, a receptor tyrosine kinase required for embryonic pattern formation, shares substrates with the sevenless and EGF-R pathways in Drosophila.

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