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The torso pathway in *Drosophila*: a model system to study receptor tyrosine kinase signal transduction

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SUMMARY

In the *Drosophila* embryo, specification of terminal cell fates that result in the formation of both the head (acron) and tail (telson) regions is under the control of the torso (tor) receptor tyrosine kinase. The current knowledge suggests that activation of tor at the egg pole initiates a signal transduction pathway that is mediated sequentially by the guanine nucleotide releasing factor son of sevenless (Sos), the $p21^{Ras1}$ GTPase, the serine/threonine kinase D-raf and the tyrosine/threonine kinase MAPKK (Dsor1). Subsequently, it is postulated that activation, possibly by phosphorylation, of a transcription factor at the egg poles activates the transcription of the terminal gap genes *tailless* and *huckebein*. These gap genes, which encode putative transcription factors, then control the expression of more downstream factors that ultimately result in head and tail differentiation. Also involved in tor signaling is the non-receptor protein tyrosine phosphatase corkscrew (csw). Here, we review the current model and discuss future research directions in this field.

Key words: *Drosophila*, signal transduction, embryogenesis, pattern formation

INTRODUCTION

In *Drosophila*, the combination of classic embryological studies and genetic analyses have uncovered four different systems that establish the body coordinates of the embryo (see reviews by Nusslein-Volhard et al., 1987; St. Johnston and Nusslein-Volhard, 1992; Fig. 1). The establishment of dorsal-ventral polarity utilizes a single patterning system, while cellular determination along the anteroposterior axis involves three independent patterning systems, the anterior, posterior and terminal systems. Determinants for each of the four patterning systems are synthesized during oogenesis and their mRNAs or proteins are localized in the egg near their prospective sites of action (Fig. 1).

The anterior and posterior patterning systems use RNA localization in the egg cell as a mechanism to generate two distinct morphogenetic gradients. The anterior system specifies the gnathal and thoracic regions and the posterior system specifies the abdominal region. The maternal mRNA for the *bicoid* (*bcd*) gene, the key member of the anterior system, is localized anteriorly in the egg and is responsible for a morphogenetic gradient that controls the domains of expression of early zygotic genes (Driever and Nusslein-Volhard, 1988a,b; Struhl et al., 1989; Finkelstein and Perrimon, 1990). Located posteriorly in the egg, the maternal *nanos* (*nos*) mRNA generates a gradient of nos protein which defines a region in which the abdominal body pattern can be specified by other morphogens (Hulskamp et al., 1989; Struhl, 1989; Irish et al., 1989; Wang and Lehmann, 1991; Barker et al., 1992).

The terminal and dorsal-ventral systems utilize a similar strategy to organize patterns; i.e., in both cases signal transduction pathways are triggered in the egg cell in response to ligands localized in the perivitelline space (Stein and Nusslein-Volhard, 1991; Sprenger and Nusslein-Volhard, 1992; Fig. 1). In each system the receptor, as well as the maternally provided downstream signal transducers, are uniformly distributed in the egg; but the signals are localized. The terminal system is required for the formation anteriorly of the acron and posteriorly of the 8th abdominal segment and the telson (as defined in Nusslein-Volhard et al., 1987). Genes of the dorsal-ventral system determine a system of positional information that results in the graded nuclear distribution of dorsal protein (Roth et al., 1989; Rushlow et al., 1989; Steward, 1989).

The establishment of terminal cell fate is under the control of a group of genes known as the terminal genes. In this paper, we review the molecules encoded by these genes and describe their respective roles in patterning. In addition, we describe the interactions of the terminal pathway with the anterior and dorsal-ventral systems that coordinately control the spatial expression of early transcription factors.

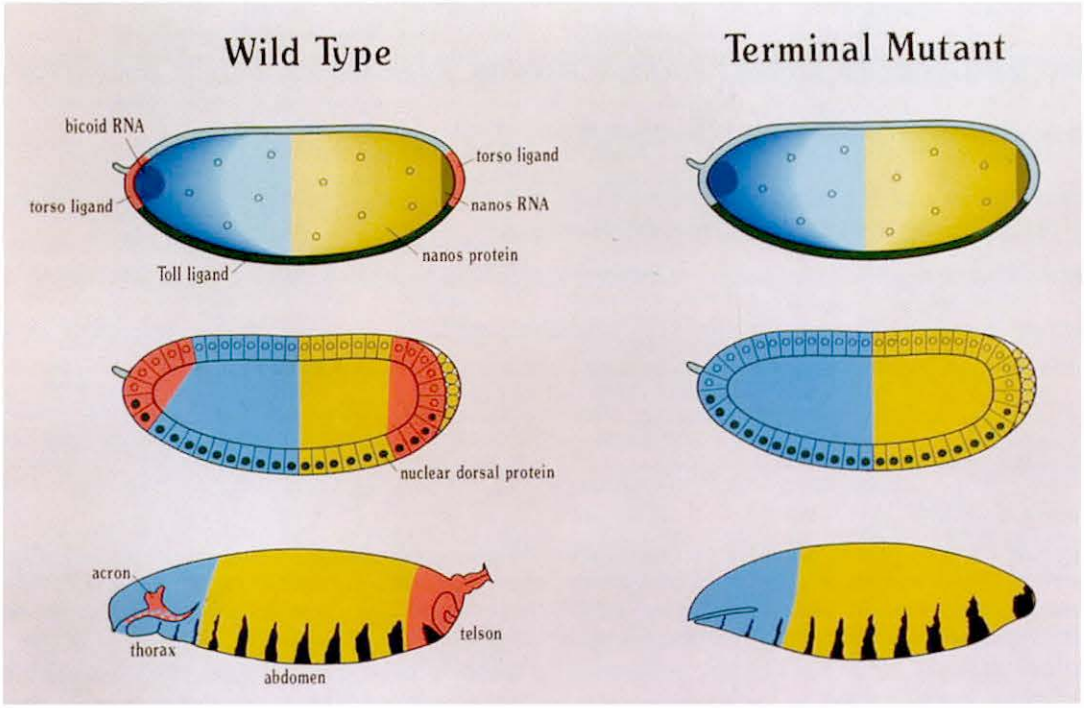


Fig. 1. The four patterning systems that determine the body axes of the *Drosophila* embryo. In the wild-type embryo, three systems operate along the anterior and posterior axes. The anterior system, a group of genes that are expressed during oogenesis, is involved in localization of maternal *bicoid* (*bcd*) mRNA at the anterior end of the egg (blue). This localized *bcd* mRNA is translated into a gradient of bcd protein in the syncytial embryo. The bcd gradient controls the expression of the anterior gap genes which specify the gnathal and thoracic regions of the embryo (blue domain). Similarly, the posterior system consists of a group of genes that are involved in localization of the maternal *nanos* mRNA at the posterior (light brown). The nos protein (yellow) specifies a region where posterior gap genes are expressed to specify the abdominal region (yellow domain). At the termini of the egg, localized torso (tor) ligand (pink) in the perivitelline fluid activates the tor RTK which ultimately triggers a phosphorylation cascade to determine the acron and telson regions (pink domains). Along the dorsal-ventral axes, Toll ligand (green) is localized in the perivitelline space at the ventral side of the egg. Activation of Toll leads to the formation of a gradient of nuclear dorsal protein (green) with the highest concentration at the ventral side of embryos where ventral fate is specified. In a terminal mutant, the signaling pathway triggered by tor RTK activation does not operate, resulting in the deletion of terminal regions and expansion of the embryonic domains specified by the bcd and nos morphogens. Anteriorly, the only cuticular structures deleted in a terminal mutant correspond to part of the head skeleton (Nusslein-Volhard et al., 1987; Ambrosio et al., 1989a). See text for a full description of the mutant phenotypes. All embryos are oriented with anterior to the left and dorsal up.

CHARACTERIZATION OF THREE GENES, *torso*, *D-raf* AND *tailless*, DEMONSTRATES THAT TERMINAL DIFFERENTIATION INVOLVES A SIGNAL TRANSDUCTION PATHWAY

Torso (tor): Mutations in the *tor* gene were first isolated in screens for recessive female sterile (fs) mutations (Schupbach and Wieschaus, 1986a). Embryos derived from mothers homozygous for *tor* null or loss of function (lof) mutations (referred to as tor^{lof} embryos) do not develop the most anterior (acron) and posterior (telson and abdominal 8 segment) terminal structures (Fig. 2B). These defects have been shown to correspond to a reorganization of the blastoderm cell fates rather than a lack of the proper differentiation of the terminal structures (Nusslein-Volhard et al., 1987; Mlodzik et al., 1987). In other words, terminal cells in tor^{lof} embryos adopt more central cell fates resulting in the deletion of entire developmental units, the terminal anlagen. Posteriorly, the deletion in a tor^{lof} embryo encompasses a region on the blastoderm fate map extending from the posterior pole (0% egg length, EL) to 20% EL. This is the region of the embryo fated to produce all structures posterior to the 7th abdominal segment, including the 8th abdominal segment, malpighian tubules, anal pads, posterior spiracles, hindgut, and posterior midgut. Anteriorly, tor^{lof} mutations affect the development of the clypeolabrum, part of the cephalopharyngeal skeleton, optic lobes and procephalic lobes (Nusslein-Volhard et al., 1987; Schupbach and Wieschaus, 1986b). Thus, the mutant phenotype of tor^{lof} embryos indicates that it is required for the specification of embryonic terminal domains.

tor encodes a putative transmembrane receptor tyrosine kinase (RTK) (Fig. 3). Neither *tor* mRNA nor protein is spatially restricted to the embryonic poles (Sprenger et al., 1989; Casanova and Struhl, 1989) suggesting that, to specify terminal development, tor is only activated at the embryonic termini. Hyperactive or gain of function (gof) tor mutations (tor^{gof}) have been isolated in which tor is ubiquitously activated in embryos independent of its ligand. In such tor^{gof} embryos, more cells adopt the terminal fate at the expense of cells of the central cell fate (Klingler et al., 1988; Strecker et al., 1989; Sprenger and Nusslein-Volhard, 1992; Sprenger

et al., 1993). Consequently, thoracic and abdominal structures are variably, or in extreme cases entirely deleted (Fig. 2C). The molecular nature of *tor* and the complementary phenotypes associated with tor^{lof} and tor^{gof} mutations suggest that tor specifies terminal cell fates through localized activation by its ligand at the embryonic poles.

D-raf (also known as *l(1)pole hole*): Mutations in the *D-raf* gene were first identified as zygotic lethal mutations associated with specific maternal effect phenotypes resembling that of tor^{lof} mutations (Perrimon et al., 1984, 1985). Identification of such mutations relies on the generation of homozygous mutant germlines in heterozygous viable females (Perrimon and Gans, 1983). Two phenotypic classes are observed among *D-raf* embryos derived from mutant germlines (Perrimon et al., 1985; Ambrosio et al., 1989a). "*D-raf* rescued" embryos, corresponding to those that do not have maternal *D-raf* activity but have received a wild-type *D-raf* gene paternally, have a phenotype identical to that of tor^{lof} embryos (Fig. 2D). "*D-raf* null" embryos, corresponding to those that do not have either maternal or zygotic *D-raf* activity and show little cuticle differentiation (Fig. 2H). Both classes of *D-raf* embryos exhibit similar defects in terminal cell fate determination as shown by the expression of early segmentation genes in these mutant backgrounds (Ambrosio et al., 1989a; Melnick et al., 1993). The poor cuticular differentiation of *D-raf* null embryos reflects the zygotic requirement of *D-raf* in other embryonic developmental processes that do not involve tor.

D-raf encodes a protein serine/threonine kinase which shows 46% identity at the amino-acid level to the human Raf-1 kinase, and as much as 65% identity at the C-terminal protein kinase (CR3) domain (Mark et al., 1987; Nishida et al., 1988; Melnick et al., 1993; Fig. 3). At the N-terminal half of the molecule, D-raf shares structural features with the human Raf-1 kinase: the cysteine zinc finger (CR1) and serine/threonine rich (CR2) regions. Like *tor* mRNA, *D-raf* mRNA is not spatially restricted to the embryonic poles (Ambrosio et al., 1989b).

Tailless (*tll*): *tll* was the first gene characterized among embryonic lethal genes that zygotically affects terminal cell fate determination (Strecker et al., 1986). Deleted in *tll* mutant embryos are structures on the blastoderm fate map from approximately 7-20% EL, including A8, the malpighian tubules, anal pads, posterior spiracles and hindgut (Fig. 2L). The posterior midgut, which originates from the most posterior region of the blastoderm fate map, is only variably affected in *tll* mutant embryos (Strecker et al., 1988). The anterior defects associated with *tll* mutations are similar to those seen in tor^{lof} embryos (Strecker et al., 1986, 1988).

The *tll* gene encodes a putative transcription factor of the steroid hormone receptor superfamily (Pignoni et al., 1990). The protein contains putative ligand binding as well as DNA binding domains (Fig. 3). In wild-type embryos at the syncytial blastoderm stage, *tll* transcripts are expressed symmetrically from 0-20% and 80-100% EL; however, by the cellular blastoderm stage, *tll* expression resolves into smaller domains with an anterior "horseshoe-like" pattern extending over the dorsal side of the embryo and a posterior domain extending from 0-15% EL (Pignoni et al., 1990, 1992). The nature of the *tll* gene product and its restricted terminal expression pattern suggests that tll specifies terminal fate by acting as a transcription factor to turn on terminal-specific gene expression.

Model

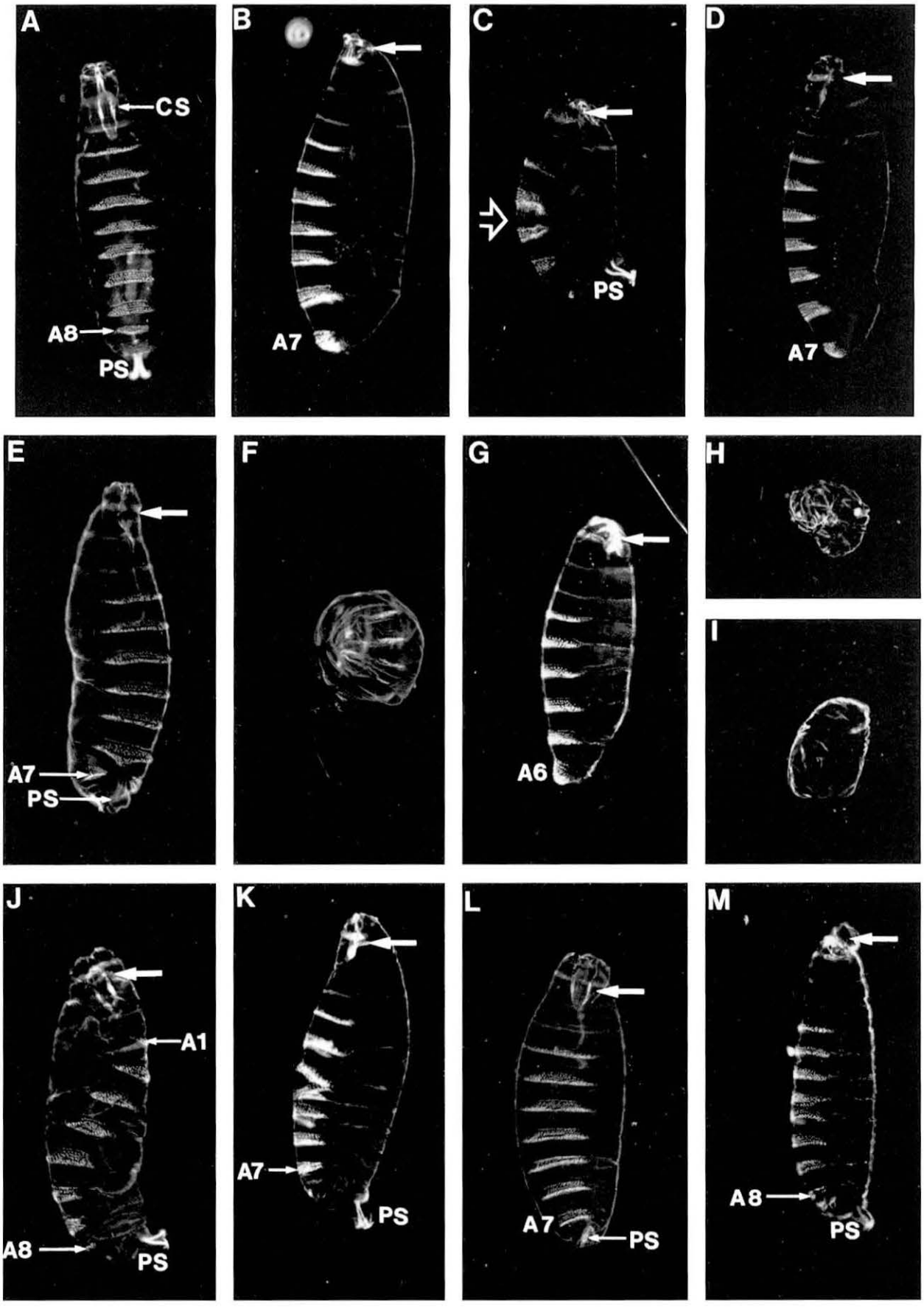
The phenotypic similarities between mutations in *tor*, *D-raf* and *tll* suggested that they may be components of the same developmental pathway that results in the differentiation of the most terminal regions of the embryo. This model was supported by the genetic epistatic analyses between tor^{gof} and either *D-raf* or *tll* loss of function mutations (summarized in Fig. 4). tor^{gof} embryos exhibit repression of thoracic and abdominal segmentation with concomitant expansion of both the acron and telson regions. Embryos derived from germlines carrying both tor^{gof} and $D\text{-}raf^{lof}$ mutations exhibit the $D\text{-}raf^{lof}$ phenotype indicating that *D-raf* activity is necessary for *tor* signaling (Ambrosio et al., 1989b). Likewise, the maternal tor^{gof} phenotype can be suppressed by the removal of zygotic *tll* function (Klinger et al., 1988). These genetic interactions indicate that the effect of tor^{gof} mutations can be negated by mutations in either *D-raf* or *tll*. These results led to the model that tor activates D-raf, which in turn controls the zygotic transcriptional activation of *tll*. Consistent with this model, the *tll* mRNA is not expressed posteriorly in either tor^{lof} or *D-raf* embryos (Pignoni et al., 1992; Melnick et al., 1993). Conversely, in strong tor^{gof} animals, *tll* is ectopically expressed along most of the anteroposterior axis of the egg (Steingrimsson et al., 1991; Fig. 4).

It should be noted that control of *tll* expression is more complex in the anterior region of the egg. In both tor^{lof} and $D\text{-}raf^{lof}$ embryos, *tll* is expressed at the anterior pole; however, it does not evolve into the wild-type horseshoe-like pattern (Pignoni et al., 1992; Fig. 4). Anteriorly, regulation of *tll* expression is under the control of the terminal, anterior and the dorsal/ventral systems (Pignoni et al., 1992; see below for further discussion).

OTHER COMPONENTS OF THE *torso* SIGNAL TRANSDUCTION PATHWAY

Genes required upstream of *torso*

Genetic screens for female sterile mutations identified four additional maternal "terminal class" genes: *fs(1)Nasrat* (*Nas*; Degelmann et al., 1986), *fs(1)pole hole* (*fs(1)ph*; Perrimon et al., 1986), *fs(2)trunk* (*trk*; Schupbach and Wieschaus, 1986a), and *fs(3)torso-like* (*tsl*; Nusslein-Volhard et al., 1987). When any one of these gene activities is missing during oogenesis, embryos develop with a tor^{lof} phenotype. The molecular nature of the products of these genes is as yet unknown; however, in genetic epistatic analyses these mutations are unable to suppress the tor^{gof} phenotype, suggesting that they function upstream of tor, and are most likely involved in the activation of the RTK. Analyses of the tissue specificity of these mutations have provided some insights into their possible roles in tor activation. Three of the upstream genes, *Nas*, *fs(1)ph* and *trk*, are germline dependent (Perrimon et al., 1986; Schupbach and Wieschaus, 1986b), while *tsl* has been shown by mosaic analysis to be required in a small number of follicle cells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located at both ends of the egg (Stevens et al., 1990). Thus, *tsl* is likely to play a key role in restricting the activation of tor at the termini (see below for further discussion).

Genes required downstream of *torso*

corkscrew (*csw*): Like *D-raf*, mutations in the *csw* gene were identified in screens for zygotic lethal mutations that exhibit a specific maternal effect on termini differentiation. Genetic epistasic analysis has shown that *csw* operates downstream of tor to positively transduce the terminal signal (Perkins et al., 1992). However, unlike *D-raf*, *csw* mutations only partially block *tor* signaling. This was determined by comparing *tll* expression in tor^{lof}, $D\text{-}raf^{lof}$ and csw^{lof} mutant embryos. Posterior *tll* expression is completely eliminated in tor^{lof} and $D\text{-}raf^{lof}$ mutant embryos (Pignoni et al., 1992; Melnick et al., 1993), whereas in csw^{lof} mutant embryos posterior *tll* expression is reduced (Perkins et al., 1992). Posteriorly, csw^{lof} embryos are missing only the posterior midgut or that part of the telson which originates from the most posterior region of the blastoderm fate map (Fig. 2J). A weak *D-raf* allele with residual activity, $D\text{-}raf^{PB26}$, also shows this phenotype (Melnick et al., 1993). Interestingly, embryos doubly mutant for both $D\text{-}raf^{PB26}$ and csw^{lof} are similar to tor^{lof} embryos, suggesting that *csw* and *D-raf* act in concert to transduce the tor signal (Perkins et al., 1992).

Molecular analysis of the *csw* gene and its products revealed that *csw* encodes a putative nonreceptor protein tyrosine phosphatase covalently linked to two N-terminal Src homology (SH) 2 domains (Perkins et al., 1992; Fig. 3). The csw protein is most similar to two mammalian proteins, PTP1C and SH-PTP2 (also known as Syp or PTP1D, Feng et al., 1993; Vogel et al., 1993) with which it shares 69 and 76% similarity, respectively (Perkins et al., 1992; Freeman et al., 1992). While the tyrosine phosphatase domain implies an enzymatic function for csw in transducing the tor signal, the presence of the SH2 domains suggest that csw effects its role by mediating heteromeric protein interactions. Both PTP1C and SH-PTP2 have been shown to associate with cytoplasmic portions of activated mammalian growth factor receptors. PTP1C associates with the EGF receptor (Shen et al., 1991) and SH-PTP2 associates, through its SH2 domains, with the EGF and PDGF receptors, as well as chimeric receptors containing the cytoplasmic domains of the HER2-neu, and kit-SCF receptors (Feng et al., 1993; Vogel et al., 1993; B. Neel, personal communication). Further genetic epistasis experiments are required to precisely place *csw* within the *tor* signaling pathway.

Ras1 and *Son of sevenless* (*Sos*): Recently, Lu et al. (1993) have devised a biochemical approach to demonstrate that the $p21^{ras}$ GTPase, encoded by *Ras1* (Fig. 3), mediates tor signaling. The approach involves the injection of either activated or dominant negative forms of putative signal transducing molecules into the early, syncytial embryos. When activated $p21^{ras}$ protein, $p21^{v\text{-}ras}$, was injected into tor^{lof} embryos, the embryos recovered their terminal structures suggesting that activation of $p21^{ras}$/Ras1 is sufficient to specify terminal identity in the absence of the tor receptor (Lu et al., 1993; Fig. 2K). This result was extended by injecting a dominant negative form of $p21^{ras}$, $p21^{rasN17}$, into wild-type embryos. In this experiment the injected embryos showed a terminal class phenotype similar to that of csw^{lof} mutant embryos. In addition, Lu et al. (1993) demonstrated that $p21^{ras}$/Ras1 functions upstream of D-raf in this pathway, since injection of activated $p21^{v\text{-}ras}$ into $D\text{-}raf^{lof}$ embryos did not show a rescuing activity.

Further support that $p21^{ras}$/Ras1 functions in the *tor* signaling pathway came from the analysis of the maternal effect phenotype of *Son of sevenless (Sos)*, where Sos^{lof} embryos derived from mutant germlines show a terminal class phenotype reminiscent to tor^{lof} and $D\text{-}raf^{lof}$ phenotypes (Lu et al., 1993; Fig. 2E,F). *Sos* encodes a guanine nucleotide releasing (or exchange) factor which is known to positively regulate $p21^{ras}$/Ras1 function by stimulating the accumulation of the active GTP-bound form of the protein (Bonfini et al., 1992; Simon et al., 1991; Fig. 3).

Dsor1 (*MAPKK*): In a screen for suppressors of a reduced activity *D-raf* allele, a gene with 65% homology to the mammalian MAP kinase kinase (MAPKK also known as MEK, Crews et al., 1992) was isolated (Tsuda et al., 1993; Fig. 3). Although this suppressor, named *Dsor1*, is a gain of function mutation, it has been shown to be a normal component of the tor pathway based on the observation that embryos derived from germlines that lack wild-type *Dsor1* activity have a terminal class phenotype (Tsuda et al., 1993; Fig. 2G,I). In tor^{lof} or $D\text{-}raf^{lof}$ mutant backgrounds, activity from the gain of function *Dsor1* mutation is sufficient to activate posterior *tll* expression, indicating that MAPKK operates downstream of both tor and D-raf. This is consistent with studies from mammalian cells where MAPKK has been shown to be a direct substrate of Raf-1 kinase (Kyriakis et al., 1992). The high level of homology of Dsor1 to mammalian MAPKK suggests the likelihood that a MAP kinase is also implicated in tor signaling.

Zygotic targets of torso signaling

In addition to *tll*, *huckebein* (*hkb*) has been shown to be

Fig. 2. The embryonic phenotypes of terminal mutants. (A) A dark field photograph of a wild-type embryo showing the well-differentiated cephalopharyngeal head sleleton (CS), thoracic and abdominal segments (A8 indicates the position of abdominal segment 8) and posterior spiracles (PS). All mutant embryos have head skeletal defects (indicated by arrows). tor^{lof} (B) and tor^{gof} (C) have complementary phenotypes: the embryo in B is missing all structures posterior to abdominal segment A7; whereas the one in C has differentiated the tail region, but abdominal and thoracic regions are reduced in number. The "rescued *D-raf*" (D), "rescued Sos^{X122}" (E) and "rescued *Dsor1*" (G) embryos show a phenotype similar to that of tor^{lof} embryos. Although "rescued *D-raf*" and tor^{lof} embryos are similar, "rescued Sos^{X122}" embryos show a weaker phenotype with only partial deletion of A8 and the posterior spiracles. "Rescued *Dsor1*" embryos have a more severe defect with only six abdominal segments being present. The "null*D-raf*" (H), "null Sos^{X122}" (F) and "null *Dsor1*" (I) embryos show very little cuticle differentiation, indicating that these genes are also involved in additional developmental processes during embryogenesis. The *csw* embryo (J) shows a twisted cuticle with all cuticlar elements present. K shows a tor^{lof} embryo injected with activated $p21^{v\text{-}ras}$ protein demonstrating the recovery of tail structures. The *tll* embryo (L) is missing A8 and part of the posterior spiracles and resembles a tor^{lof} embryo. In *hkb* (M) mutant embryos all cuticular elements are present. See text for a description of the effects of each mutation on internal structures.

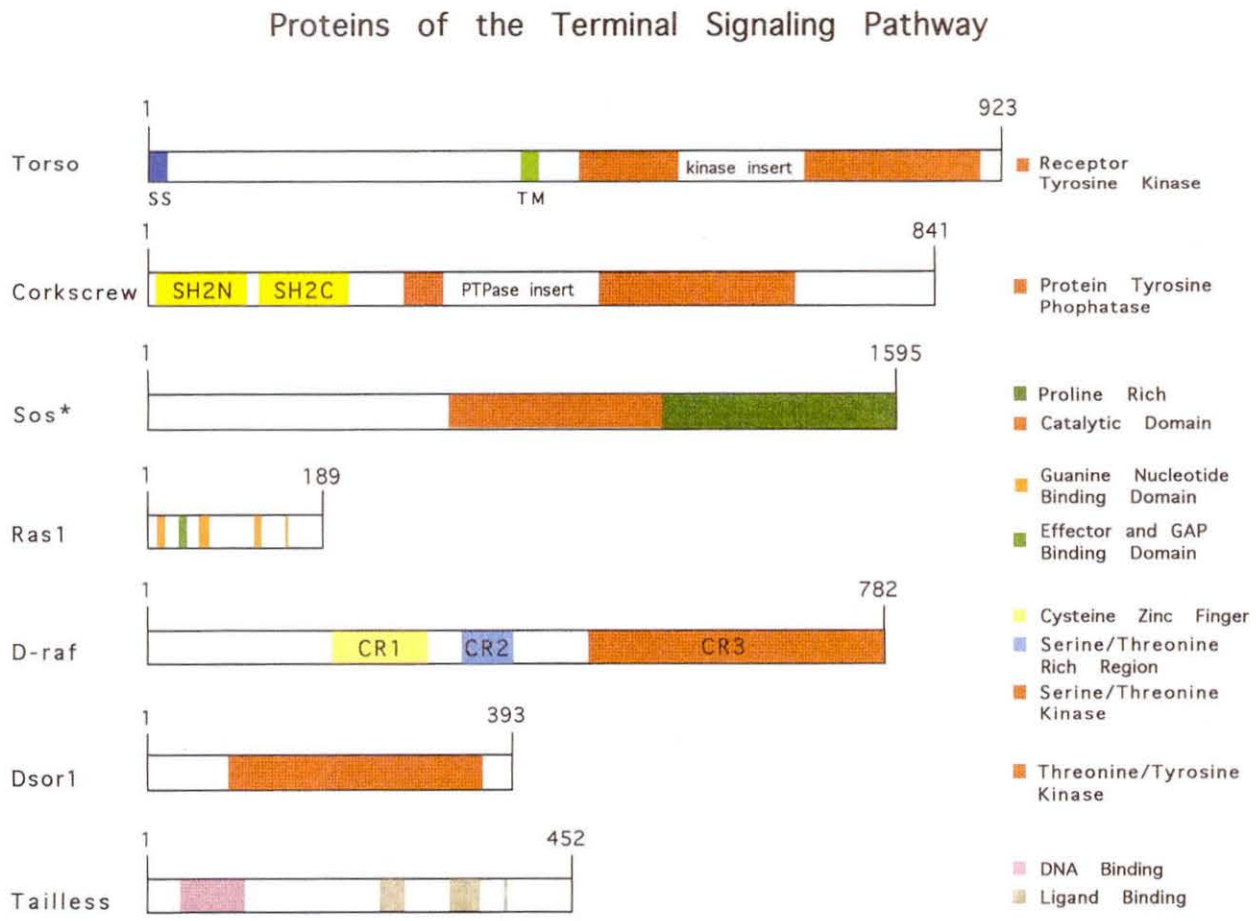

Fig. 3. The proteins encoded by the terminal genes. Schematized are the various proteins encoded by the genes described in the text which outlines their main features. *Note: The protein encoded by *Sos* is not represented at the same scale as the others.

another zygotic gene that responds to tor signaling (Weigel et al., 1990). In wild-type, *hkb* transcripts are expressed in caps at both anterior and posterior ends of the blastoderm stage embryo. Posteriorly, this expression extends from 0 to 12% EL, or that region destined to form the posterior midgut (Bronner and Jackle, 1991). Posteriorly, in *hkb* mutant embryos only the posterior midgut is deleted (Weigel et al., 1990; Fig. 2M), which is the only posterior structure not completely deleted in *tll* mutant embryos. Both the co-extensive nature of the *tll* and *hkb* mutant phenotypes and the observation that *tll-hkb* double mutant embryos are phenotypically similar to tor^{lof} mutant embryos (Weigel et al., 1990) suggests that posteriorly *tll* and *hkb* are sufficient to mediate the maternal activity of *tor*. The *hkb* gene has been shown to encode a putative transcription factor with multiple zinc fingers (H. Jackle, personal communication).

The terminal gap genes *tll* and *hkb* control the expression of more "downstream terminal" genes. Presently, these include the transcription factors: *hunchback* (Tautz, 1988), *fork head* (Weigel et al., 1990), *fushi-tarazu* (Mlodzik et al., 1987), the regulatory element of the homeotic gene *Abdominal B* (Casanova et al., 1986), and *Kruppel* (Rosenberg et al., 1986). These genes are expressed at the posterior terminus of the embryo and their expression patterns are perturbed in embryos mutant for upstream terminal genes (see review by Perkins and Perrimon, 1991). These transcription factors most likely control the expression of further downstream genes involved in cellular differentiation of terminal structures.

THE CURRENT MODEL OF TORSO SIGNAL TRANSDUCTION PATHWAY

Fig. 5 summarizes our current understanding of the tor signaling pathway. Four genes, *tsl*, *trk*, *Nas* and *fs(1)ph*, operate upstream of *tor* and are probably involved in the production of an active tor ligand. Undoubtedly, the four loci encode only a subset of the proteins required for the generation, transport and secretion of the tor ligand. Three of these loci are required in the germline, while one (*tsl*) is required in the surrounding follicle cells, suggesting that intercellular communication is required to activate tor.

The molecular nature of the tor ligand remains elusive. However, Sprenger and Nusslein-Volhard (1992) have shown that it is diffusible in the perivitelline space and present in limited quantity at the egg poles. Because the tor RTK is uniformly distributed in the egg membrane, a limited amount of the diffusible ligand originating from the termini is crucial for the activation of tor within the embryonic terminal domains where terminal cell fates become specified. *tsl*, because of its requirement in a small number

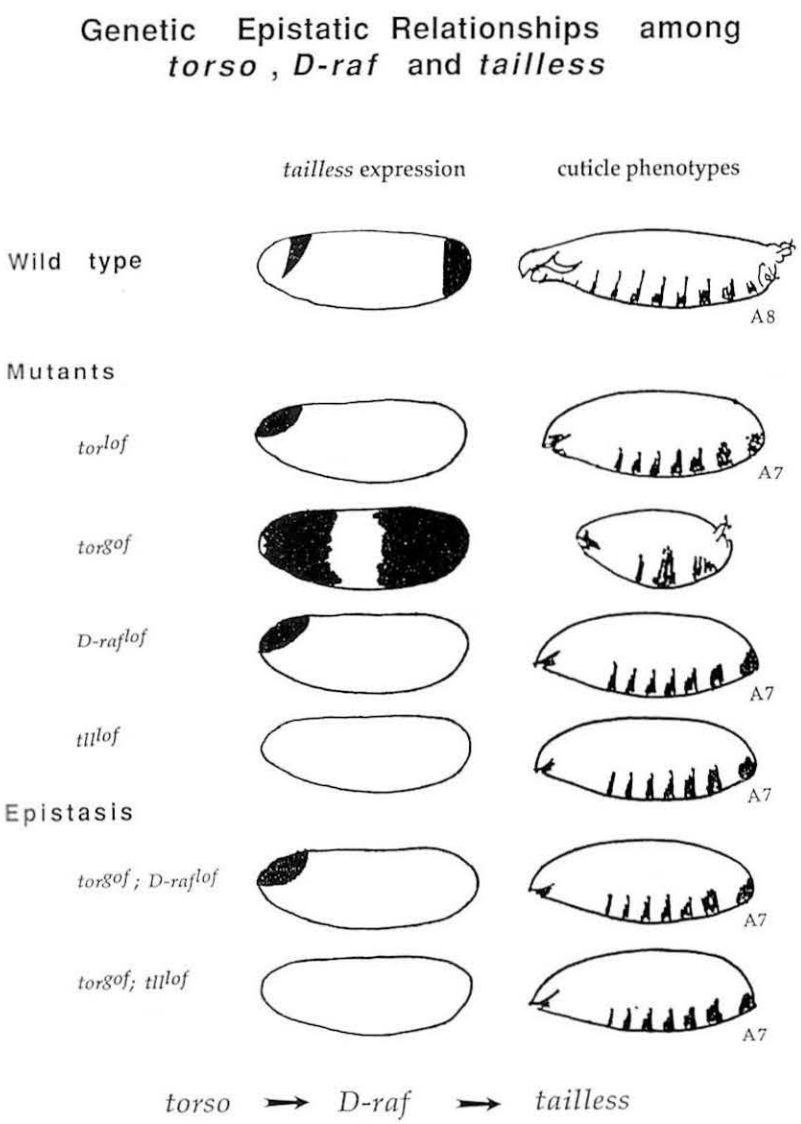

Fig. 4. Genetic epistatic relationships among *torso*, *D-raf* and *tailless*. The genotypes listed in the left column are maternal genotypes with the exception of tll^{lof} which is the homozygous zygotic genotype. The middle column are cellular blastoderm stage embryos showing the *tll* expression patterns in various mutant backgrounds. The corresponding cuticular phenotypes are shown in the right column. A combination of the genetic epistatic analyses and the altered *tll* expression in mutant embryos suggests that all three genes affect the same developmental process, where *tor* lies upstream of *D-raf* which is upstream of *tll*.

of follicle cells located at both ends of the oocyte, can either encode the tor ligand, or alternatively be involved in the activation of the tor ligand (Stevens et al., 1990). In the first case, an inactive form of tsl protein may be localized to the terminal regions of the vitelline membrane prior to fertilization. Following fertilization, secretion into the perivitelline space of an activating molecule, possibly encoded by one of the three germline dependent maternal genes, will lead to the release of a diffusible activated tsl protein. In the second case, following fertilization an inactive form of the tor ligand may be secreted into the perivitelline fluid, and subsequently become activated by a molecule (possibly encoded by *tsl*) localized to the terminal regions of the vitelline membrane.

Activation of tor triggers a signal transduction pathway mediated by the proteins Sos and Ras1 which in turn activates a phosphorylation cascade through the protein kinases D-raf and MAPKK (Dsor1). Currently, the transducers of tor and their regulatory mechanisms are similar, if not identical, to those found for other RTKs; e.g. Sevenless (sev) in *Drosophila* (Simon et al., 1991; Dickson et al., 1992a), and PDGF and EGF growth factor receptors in mammals (reviews by Ullrich and Schlessinger, 1990; Cantley et al., 1991). The guanine nucleotide releasing factor, Sos, transduces the signals generated by both tor and sev to Ras1, and activation of Ras1 is sufficient to initiate either terminal development in early embryos or R7 cell differentiation in the eye imaginal disc (Fortini et al., 1992; Lu et al., 1993). Dickson et al. (1992b) have shown that for the development of the R7 photoreceptor cell the kinase domains of either tor and sev are functionally equivalent. This indicates that the specificity of different RTKs is likely determined by the developmental potential of the cells in which they are activated. The activation of *tll* and *hkb* in early embryos may be explained by the availability of a specific transcription factor, postulated as *gene Y* (St. Johnston and Nuslein-Volhard 1992) which, once phosphorylated in response to tor signaling, activates transcription from the *tll* and *hkb* promoters.

Little is known about how the overlapping spatial domains of *tll* and *hkb* expression are achieved. Previous studies (Casanova and Struhl, 1989) have proposed that

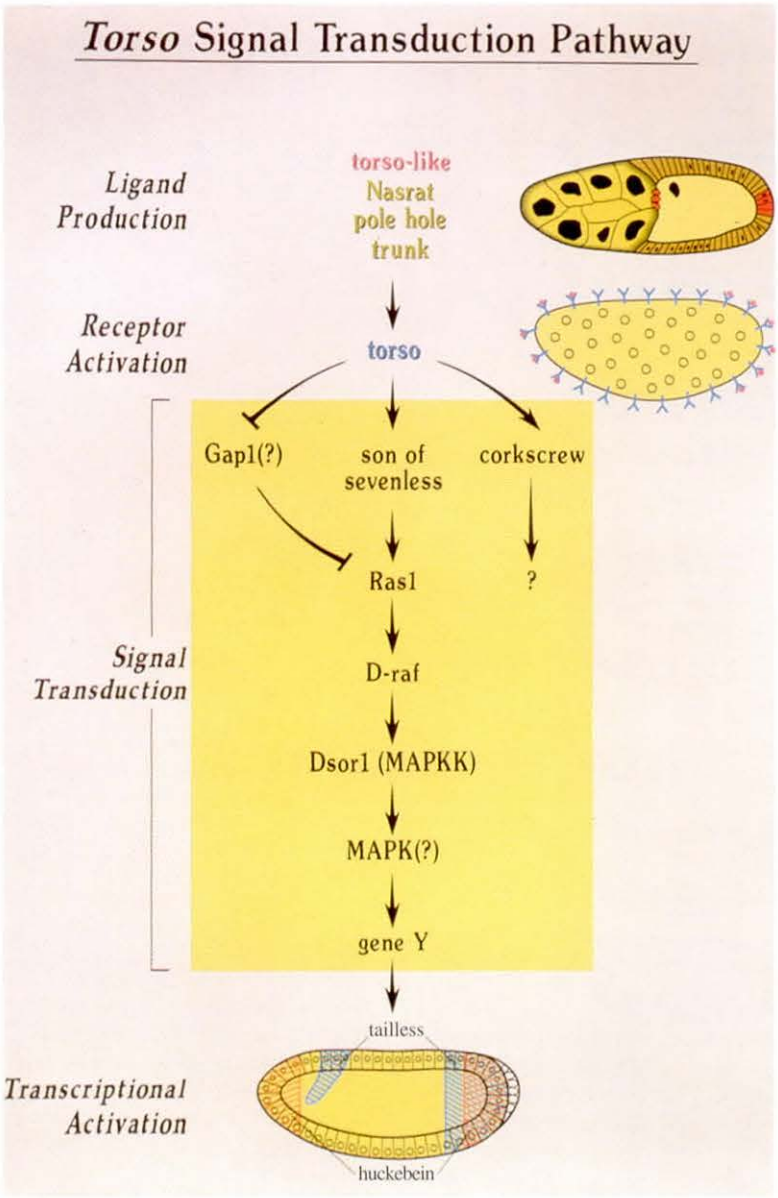

Fig. 5. The current model of the torso signaling pathway. → indicates activation steps and ⊣ indicates a negative regulatory steps. Three genes *Gap1*, *MAPK* and *gene Y* are postulated to operate in tor signaling (see text). *Gap1*, which encodes a putative Ras GTPase activating protein (Gaul et al., 1992) may play a role in this pathway as a negative regulator of tor signaling (Lu et al., 1993). MAPK (MAP-kinase) is a postulated member of the tor signaling pathway based on its known involvement downstream of MAPKK in mammalian RTK signaling pathways (see review by Pelech and Sanghera, 1992). *Gene Y* is postulated to be a transcription factor required for the initiation of transcription of the terminal gap genes *tll* and *hkb*. See text for a detailed description of the tor signaling pathway. Note that the *fs(1)pole hole* which is involved in ligand production and *D-raf* (= *l(1)pole hole*) are two different genes that should not be confused.

graded levels of tor signaling are required to direct pattern formation within the posterior unsegmented terminal anlagen (0-20% EL). This gradient of tor activation is thought to be generated by diffusion of localized tor ligand originating from the poles. However, we have observed that posterior terminal structures were rescued at their normal posterior position even when activated $p21^{ras}$/Ras1 was injected anteriorly or in the central region of tor^{dof} embryos. These results suggest that embryos devoid of the tor RTK still possess an intrinsic polarity for tail formation. This polarity arises independently of tor activation somewhere downstream of $p21^{ras}$/Ras1. These observations are consistent with the recent postulation by Sprenger and Nusslein-Volhard (1992) that "activated tor might act more like a switch, triggering terminal development after a threshold level of tor activity is achieved", and "gradient(s) of cytoplasmic molecules" would then be responsible for subdividing the terminal anlagen.

INTERACTION AMONG THE TORSO SIGNALING, THE BICOID AND THE DORSAL/VENTRAL SYSTEMS

Even though the four maternal patterning systems are separable genetically, they interact at the molecular level. For cells at the termini, three systems (anterior or posterior, terminal and dorsal-ventral) converge to establish the positional values of individual nuclei. These interactions have been especially studied at the anterior end. Many genes activated by bicoid (bcd), such as *orthodenticle* and *hunchback*, are repressed in the anterior tip by the tor signaling pathway (Tautz, 1988, Finkelstein and Perrimon, 1990). This is also the case for *snail*, a gene activated by the dorsal (dl) morphogen gradient on the ventral side of the embryo (Casanova, 1990; Ray et al., 1991). Similarly, the anterior *tll* expression pattern is also influenced by the anterior (bcd) and dorsal-ventral (dl) systems. Pignoni et al.

(1992) have shown that the anterior "horseshoe-like" pattern of *tll* expression at the late blastoderm stage is the result of positive activation by intermediate levels of bcd and tor activities plus repression at a high level of tor, bcd and dl concentrations.

PERSPECTIVES OF THE TERMINAL SYSTEM

We have described the molecular nature and roles of each of the known components involved in the tor signaling pathway. There are certainly more terminal genes that remain to be identified and one approach to identify these genes is to pursue the isolation of mutations associated with terminal defects. Although genetic screens for recessive female sterile and embryonic lethal mutations are near saturation, previous searches for zygotic lethal mutations associated with specific maternal effect phenotypes have so far only been conducted for the X-chromosome (Perrimon et al., 1989; Chou and Perrimon, 1992). We expect that many other genes will be identified, as were *D-raf* and *csw*, once screens to detect the maternal effects of essential genes are extended to the remaining of the genome. Another powerful approach involves the isolation of mutations that modify (by suppression or enhancement) the phenotypes of a pre-existing mutations. For example, *Dsor-1* was identified as a supressor of a weak *D-raf* allele (Tsuda et al., 1993). Such interaction screens allow one to isolate essential genes with multiple functions throughout development, because the inactivation of one copy of the gene is often enough to show interactions in a sensitized genetic backgound (Simon et al., 1991). Finally, complementary approaches such as the injection into embryos of various molecules can be used to test their putative roles in tor signaling. Good candidates for this approach include previously identified components of other RTKs signaling pathways.

Once new components are identified, it will be important to address their necessity and sufficiency in signaling. Such analysis, in combination with detailed biochemical analysis, will allow a full understanding of the role each component plays in tor RTK signaling. The tor signaling pathway, with its unique opportunity to combine genetic, cytoplasmic injection of molecules and biochemical approaches, provides an excellent model system not only to identify the components involved in RTK signaling but also to understand the epistatic and biochemical relationships between the various signaling molecules.

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