Signalling pathways initiated by receptor protein tyrosine kinases in *Drosophila*

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The isolation and characterization of *Drosophila* mutations in receptor protein tyrosine kinases (RPTKs) have allowed a detailed analysis of the cellular processes regulated by these proteins. Recent investigations have identified a number of putative ligands involved in the activation of the receptors, and have demonstrated that these RPTKs trigger an evolutionarily conserved biochemical pathway. In addition to molecules previously identified from vertebrate studies, i.e. Grb2, Sos, Ras-Gap, $p21^{ras}$, Raf, MEK and MAPK, genetic studies have suggested that two novel proteins, the protein tyrosine phosphatase (PTPase) Csw and the transmembrane protein Rho, are involved in RPTK signalling.

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Introduction

The functions and mechanisms of action of RPTKs have been a major focus of research on *Drosophila* in the past few years. To date, seven *Drosophila* RPTKs have been identified: Sevenless (Sev), Torso (Tor), *Drosophila* epidermal growth factor receptor (DER), *Drosophila* fibroblast growth factor receptor (DFR)-1, Breathless (Btl), *Drosophila* trk (Dtrk) and *Drosophila* insulin receptor homologue (DIRH) (see Fig. 1 for the structure of these RPTKs and references), and mutations in four of them (Sev, Tor, DER and Btl) have enabled the establishment of model systems for analyzing the roles of RPTKs in the control of cellular processes such as the regulation of cell growth, differentiation, migration and viability.

The analysis of the mutant phenotypes associated with *Drosophila* RPTKs has been key in addressing their instructive abilities. For example, these studies have defined the roles of Sev, Tor and DER in determinative events; have demonstrated that DER is involved in the control of cellular division; and have implicated Btl in the control of cellular migration. In addition, the availability of *Drosophila* RPTK mutations has allowed their signalling pathways to be genetically dissected, leading to the identification of many components involved in either the activation of the RPTK or the transduction of the signal from the membrane to the nucleus. These studies have contributed to the realization that RPTKs activate a biochemical signalling pathway which has been conserved throughout evolution [1–3].

The methodologies used to study *Drosophila* RPTKs rely on genetic techniques rather than biochemical approaches, in contrast with studies on vertebrate RPTKs. The genetic approach is based on three working hypotheses: first, mutations having related mutant phenotypes most likely identify genes that encode molecules involved in the same biochemical pathway [4]; second, extragenic modifiers of either a gain-of-function or a reduced-activity mutation identify gene functions that participate in the same signalling pathway [5,6•]; and third, a null mutation in a gene that operates downstream of another gene should suppress the effect of an activating mutation in a more upstream component [7,8•].

In this review, I describe the cellular roles of each of the known *Drosophila* RPTKs, and further, how genetic methodologies have been applied to the dissection of RPTK signalling pathways. In addition, I discuss the role of two recently identified genes (*csw* and *rho*) in RPTK signalling.

Sevenless and photoreceptor R7 development

Studies on the compound eye have provided a paradigm for the study of inductive interactions in the determination of cell fates [9]. The Sev RPTK is required in a cell-autonomous manner for cell-fate determination of only one of the eight photoreceptor cells, the

Abbreviations

Boss—Bride of sevenless; **Btl**—Breathless; **CNS**—central nervous system; **Csw**—Corkscrew; **DER**—*Drosophila* epidermal growth factor receptor; **DFR**—*Drosophila* fibroblast growth factor receptor; **DIRH**—*Drosophila* insulin receptor homologue; **Grk**—Gurken; **MAPK**—mitogen-activated protein kinase; **MEK**—MAPK kinase; **PTPase**—protein tyrosine phosphatase; **Rho**—Rhomboid; **RPTK**—receptor protein tyrosine kinase; **Sev**—Sevenless; **Sos**—Son of sevenless; **Spi**—Spitz; **Tor**—Torso; **Tsl**—Torso-like.

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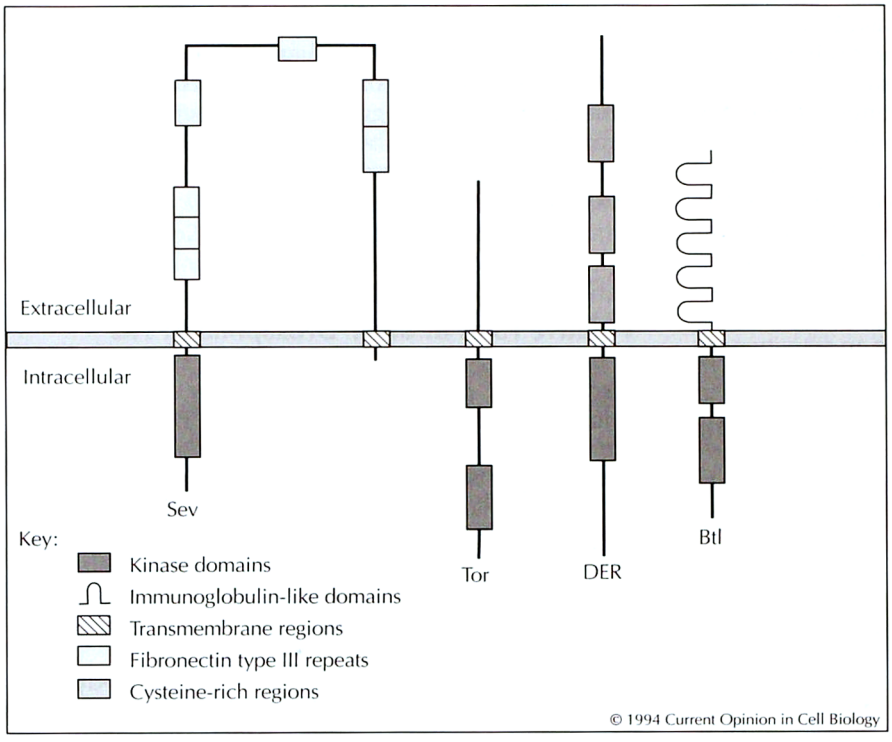

Fig. 1. Structure of *Drosophila* RPTKs whose mutations have been isolated. Structures of Sev, Tor, DER and Btl. All RPTKs have a similar structure that consists of three different domains: an extracellular domain responsible for ligand binding, which is connected through a membrane-spanning region to a cytoplasmic tyrosine-kinase domain. Sev is synthesized as a precursor that is cleaved into two subunits that remain associated by non-covalent interactions [65]. Tor RPTK contains a split tyrosine-kinase domain in the intracellular domain, and its structure is reminiscent of the mammalian platelet-derived growth factor receptor [18,20]. DER is similar to the mammalian epidermal growth factor receptor [29,62]. Btl [34,37] contains five Ig-like domains in the extracellular domain and a split tyrosine-kinase domain. For the structures of DFR1, Dtrk and DIRH, see [34,36,63], respectively. Adapted from [64].

R7 cell, in each eye ommatidium. In a *sev* mutant, R7 fails to differentiate and instead becomes a lens-secreting cone cell. Even though its mutant phenotype is limited to only one cell type, *sev* is expressed transiently in eight of the 20 cells of the ommatidium: R3, R4, R7, the mystery cell and the cone cells [10]. This broad domain of expression suggests that Sev specificity must be regulated by a more localized signal. The transmembrane protein Bride of sevenless (Boss), which is required by R8 for R7 development, is thought to encode a ligand for Sev [11,12]. This model is further supported by the following four findings. First, *boss* expression is restricted to R8. Second, heterotypic cell aggregates can be formed between *boss*- and *sev*-expressing cell lines. Third, Boss is internalized in a Sev-dependent manner [13]. Fourth, *boss* is instructive in promoting R7 cell fate, as ubiquitous expression of *boss* induces the cone cells to become R7 cells [14].

Expression of activated forms of *sev* has revealed that expression of the fate of the R7 cell depends on the activation of the Sev RPTK pathway, as well as on the cell's history. Expression of activated forms of *sev*, either in *sev*-expressing cells [15] or ubiquitously [16], induces the mystery and cone cells to become R7 cells, but no transformations of the R3 and R4 photoreceptor cells are observed. These results indicate that only some of the *sev*-expressing cells are competent for neuronal induction by Sev activation. Possibly, some of the components necessary for transduction of the signal received by Sev are co-expressed with *sev*, and thereby define a pre-existing pattern of developmental potential [16]. In addition, the observation that activated Sev in R3 and R4 cells does not induce these cells to enter the R7 fate path indicates that these cells become limited in their developmental potentialities very early in ommatidial development. This may be a necessary prerequisite for proper development of ommatidia, as these cells also contact R8.

Torso and differentiation of the embryonic termini

Genetic analyses of the maternal systems that control the determination of the embryonic plan have identified one system, the terminal system, that organizes the formation of both the most anterior and posterior regions of the embryo [3,4,17]. This system is under the control of the Tor RPTK, whose kinase activity is both necessary and sufficient for terminal differentiation [18,19••,20]. Tor protein is not spatially restricted to the embryonic poles [21], suggesting that an activating factor is localized at each terminus of the egg. The Tor ligand is most likely localized in the extracellular space at both embryonic termini and is limited in amount [19••].

Genetic analysis of other mutations associated with terminal phenotypes identified the product of the gene *tsl* (*torso-like*) as a possible candidate for a Tor ligand. In genetic epistasis experiments, *tsl* was found to act upstream of *tor* [22]. In addition, mosaic analyses of Tsl showed that its activity is required only in a subset of specialized follicle cells located at the termini of the egg chamber [23]. Molecular characterization of *tsl* [24••] revealed that it encodes a novel protein that is likely to be secreted. During oogenesis, *tsl* transcripts

are found in the follicle cells located at both ends of the egg chamber. These follicle cells most likely secrete Tsl into the perivitelline space, which, by a mechanism not yet understood, leads to the terminal activation of Tor [24••]. Further indication that Tsl specifies the regions in which Tor becomes activated was obtained from ectopic expression of *tsl*. Ubiquitous expression of *tsl* during oogenesis leads to a phenotype reminiscent of gain-of-function *tor* mutations, in which abdominal segmentation is repressed and terminal regions are expanded [24••]. Biochemical experiments are needed to conclusively demonstrate that *tsl* encodes the Tor ligand; however, it is clear that *tsl* encodes an activity necessary and sufficient for Tor activation.

Multiple roles of DER during development

In contrast to Sev and Tor, DER is broadly expressed [25] and performs multiple functions during *Drosophila* development. During embryogenesis, DER is involved in the establishment of ventral cell fates, survival of amnioserosa and ventral ectodermal cells, CNS development, production of embryonic cuticle, and germ-band retraction [26–28]. During imaginal development, DER is involved in a variety of developmental processes including imaginal cell proliferation and wing vein formation [26]. During oogenesis, DER is involved in the determination of the dorsal–ventral polarity of the egg [29].

Genetic analyses of DER support the model in which the multiple roles associated with DER are associated with multiple receptor activities. Some DER mutations affect specific developmental processes; for example, there exists a set of DER mutations referred to as *torpedo* that exhibits only the oogenesis phenotype [29]. In addition, positive and negative interactions can be observed between various DER mutations, suggesting that DER is regulated by multiple ligands [26,30].

Recently, support for the model that DER is associated with multiple receptor activities has been obtained from the characterization of two putative ligands for DER, Spi [31••] and Grk [32••]. Both the *spi* and *grk* genes encode molecules similar to TGFα, which has been shown to biochemically activate the vertebrate epidermal growth factor RPTK (for review, see [33]). The torpedo mutations lead to a ventralization of egg chambers that is similar to the *grk* phenotype. Unlike *torpedo*, whose activity is required in the follicle cells, *grk* activity is required in the germ line. During oogenesis, *grk* transcripts accumulate asymmetrically, at the dorsal corner of the oocyte. This distribution most likely results in the production of a spatially restricted ligand, which, when secreted into the perivitelline space, activates DER in the dorsal follicle cells [32••].

During embryogenesis, one DER ligand is likely to be encoded by the *spi* gene. The spi mutant embryos exhibit a subset of the defects seen in DER mutant embryos [26,27,31••]. These defects are associated with dorsal–ventral axis formation, glial cell migration, sensory organ determination and muscle development. Both Spi and DER are broadly expressed during embryonic development, suggesting that other cues, possibly provided by Rho (see below), are responsible for determining the specificity of the Spi–DER ligand–receptor interaction.

Putative roles of other *Drosophila* RPTKs

The cellular processes controlled by the remaining known *Drosophila* RPTKs, DFR1, Btl, Dtrk and DIRH, have yet to be studied in detail. On the basis of their expression patterns, however, it has been proposed that DFR1, DIRH and Dtrk play a role in mesoderm development, neurite outgrowth and neural development, respectively [34–36]. DFR1 is expressed first in the presumptive embryonic mesoderm and at later stages in muscle precursor cells [34]. Dtrk, which shares structural homology with neural cell adhesion molecules of the immunoglobulin superfamily, is expressed dynamically during development of the CNS, where it may regulate neuronal recognition [36]. DIRH mRNA are maternally stored and localized uniformly in early embryos. During later embryonic development, DIRH expression increases in the CNS at the time corresponding to the period of active neurite outgrowth, suggesting a possible role for DIRH in this process [35].

More is known about the function of *btl*. During embryogenesis, *btl* is expressed in invaginating endodermal, mesectodermal and epidermal cells [34,37]. Analysis of *btl* mutations [38••] has indicated a role in cell migration for this RPTK, because subsets of glial cells fail to migrate to their proper position during embryonic CNS formation in *btl* mutant animals. In addition, the embryonic tracheal tree does not differentiate properly in *btl* mutant animals and exhibits a phenotype that has been associated with defective tracheal-cell migration, but not with division and differentiation.

Drosophila RPTKs activate the same biochemical pathway

Studies on vertebrate, *Caenorhabditis elegans* and *Drosophila* RPTKs have converged on a common pathway triggered by different RPTKs (see recent reviews by Williams [1], Egan and Weinberg [2] and Perrimon [3]; Table 1). Following ligand binding, RPTKs dimerize, which presumably induces transphosphorylation of specific tyrosine residues on the cytoplasmic domain of the receptors. These phosphotyrosines then create multiple binding sites for target cytoplasmic proteins, which bind to the activated receptor through their SH2 domains. One of these binding proteins, Drk (also known as Sem 5 in *C. elegans* and Grb2 in vertebrates),

has recently been implicated in Sev [39••,40••] and Tor [6•] signalling. Drk contains one SH2 and two SH3 domains. Biochemical analyses have revealed that the SH2 domain of Drk binds to the activated Sev receptor, and that the SH3 domains bind to the guanine releasing factor protein Sos [39••,40••,41]. Activated Sos facilitates the GDP/GTP exchange on $p21^{ras}$/Ras1, which further transduces the signal. Consistent with these observations, Sos and $p21^{ras}$/Ras1 have been implicated in the Sev [5,42,43•] and Tor [6•,44•] signalling pathways. In addition, a negative regulator of $p21^{ras}$/Ras1 encoded by Gap-1 has been implicated in Sev [45] and DER [46] signalling.

In vertebrate cells, an increase in the GTP-bound form of $p21^{ras}$/Ras1 results in the activation of the Raf–MEK–MAPK kinase cascade pathway [2]. Consistent with results of vertebrate studies, the *Drosophila* homologue of the mammalian Raf-1 serine/threonine kinase has been implicated in the Tor [7,47], Sev [8•] and DER [48,49•] pathways. On the basis of biochemical studies conducted in vertebrate cells, activated Raf-1 in turn positively activates MEK, a tyrosine/threonine kinase. Recently, the gain-of-function mutation *Dsor-1* was isolated in a *Drosophila* MEK gene during a search for second-site suppressors of a weak *D-raf* allele [50••]. *Dsor1* is able to suppress the terminal defects associated with mutations in *D-raf* as well as in more upstream components. Further demonstration of the role of this MEK in Tor signalling was obtained from the analysis of loss-of-function mutations in this gene, which have maternal-effect phenotypes similar to both *tor* and *D-raf* mutations.

The nature of the downstream components of MEK remains to be elucidated. Studies in both vertebrate and yeast cells have shown that MEK activates a MAPK that directly phosphorylates transcription factors [51,52]. In *Drosophila*, a MAPK has been isolated [53]; however, no mutations are as yet available, so how the RPTK-generated signals are transduced from the cytoplasm to nuclear factors remains unclear. The nature of the transcription factors that are direct targets of MAPK still remains to be characterized. In the Tor system, no candidates have yet been isolated [3]. In the Sev pathway, however, a putative DNA-binding protein, Yan [54], which contains multiple putative MAPK phosphorylation sites, is a good candidate for one of these factors.

Role of Corkscrew in RPTK signalling

Genetic analyses of embryonic development have identified a non-receptor PTPase, Corkscrew (Csw) [55••], as a member of the Tor signalling pathway. Null mutations in *csw* are associated with a terminal phenotype reminiscent of weak *D-raf* mutations [48]. Genetic epistasis analyses have shown that Csw activity is needed downstream of Tor, because loss-of-function *csw* mutations suppress the dominant phenotype of *tor* gain-of-function mutations [55••]. In addition, Csw may regulate the activity of $p21^{ras}$/Ras1, as over-expression of an activated $p21^{ras}$ protein in *csw* mutant animals can rescue aspects of the *csw* mutant phenotypes [44•].

Csw is most similar to the mammalian Syp protein (also known as SH-PTP2 or PTP1D; [56–58]). The biochemical roles of these PTPases in RPTK signalling are unknown; however, the presence of two SH2 domains in these proteins suggests that they could directly bind the activated RPTK. This model is supported by recent studies with Syp, which physically associates with various RPTKs and which becomes tyrosine phosphorylated upon binding [57,58].

Table 1. Molecules involved in *Drosophila* RPTK signalling.

	Ligand		
Signal cascade proteins	Tsl	Boss	Spi, Grk
RPTK	Tor	Sev	DER
Adaptor (Grb2)	Drk	Drk	nd
PTPase (Syp)	Csw	nd	nd
GRF	Sos	Sos	nd
GAP	nd	Gap1	Gap1
GTPase ($p21^{ras}$)	Ras1	Ras1	Ras1
Ser/Thr kinase	D-raf	D-raf	D-raf
Thr/Tyr kinase (MEK)	Dsor1	nd	Dsor1
Ser/Thr kinase (MAPK)	nd	nd	nd

GAP: GTPase-activating protein; GRF: guanine-nucleotide releasing factor; nd: proteins whose function in a specific RPTK pathway have not yet been determined.

Role of Rhomboid in DER RPTK signalling

A putative transmembrane protein encoded by *rhomboid* (*rho*) [59] may play a key role in controlling the spatial activation of DER. The expression pattern of *rho* is extremely dynamic, and correlates well with the domains where DER activity is required. For example, during mid-stages of oogenesis, *rho* is expressed on the apical surface of the dorsal-anterior follicle cells [60••], which require DER activity for normal differentiation [32••]. Lack of *rho* activity in these follicle cells affects their normal cell fates and ventralizes the egg chamber, a phenotype reminiscent to lack of either *grk* or *DER/torpedo* activities [60••]. The instructive ability of *rho*, analyzed by ectopic expression experiments, has shown that ubiquitous expression of *rho* in follicle cells leads to dorsalization of the egg chambers [60••]. Interestingly, this dorsalization is dependent upon Grk and DER activities, suggesting that Rho may play a role in restricting the activation of DER.

Such a cooperative relationship between Rho and DER has also been suggested from the analysis of *spi*. Similar embryonic requirements are shared by *spi*, *DER* and *rho* [26,27,31••,59]. Because *spi* and DER are broadly expressed during embryogenesis and because *rho* is restricted to cells which require its function, it has been suggested that Rho may provide a cue necessary for the Spi–DER ligand–receptor interaction [31••].

Similar conclusions have been drawn from the studies of Rho in wing vein development [61••]. Expressed in the presumptive wing veins, *rho* is both necessary and sufficient for vein formation, a patterning process that also requires DER activity. Gene-dosage studies indicate that localized expression of Rho may amplify the signalling pathway activated by the ligand–DER interaction.

Conclusions

Recent studies on *Drosophila* RPTKs have revealed many insights into the developmental decisions controlled by RPTKs, the mechanisms of activation of the receptors, and the nature of the biochemical pathways that are activated by the RPTKs. In particular, studies on Sev, Tor and DER have shown that the domains of expression of these RPTKs are broader than the sites in which they are required. Precise activation of the RPTK signalling pathways requires interactions with ligands whose spatial and temporal expression is tightly regulated. Possibly, other factors such as Rho, whose biochemical activity remains to be characterized, are also involved in regulating the ligand–receptor interaction.

The genetic dissections of RPTK signalling pathways in *Drosophila* have identified proteins previously unidentified from biochemical analyses as important signal transducers. The existence of a universal 'signalling cassette' that operates downstream of RPTKs raises important questions regarding the specificity of signalling. The array of transcription factors available in different cell types appears to be the main determinative factor in cell-fate determination. Further dissection of RPTK signalling pathways will demonstrate whether this concept is correct.

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