Signalling pathways initiated by receptor protein tyrosine kinases in Drosophila

Norbert Perrimon

Howard Hughes Medical Institute, Boston, USA

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, p21ras, 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.

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...
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 seu-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 extra-cellular space at both embryonic termini and is limited in amount [15**].

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 terminus 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 TGFβ, 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 mesodermal 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 phenotypic 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 p21ras/Ras1, which further transduces the signal. Consistent with these observations, Sos and p21ras/Ras1 have been implicated in the Sev [5,42,43] and Tor [6,44] signalling pathways. In addition, a negative regulator of p21ras/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 p21ras/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 Usor-1 was isolated in a Drosophila MEK gene during a search for second-site suppressors of a weak D-raf allele [50]. Usor-1 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 p21ras/Ras1, as over-expression of an activated p21ras 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.

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<th>Signal cascade proteins</th>
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<td>Tsl</td>
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<td>RPTK</td>
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<td>Adaptor (Grb-2)</td>
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<td>PTPase (Syp)</td>
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<td>GTPase (p21ras)</td>
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<td>Ser/Thr kinase</td>
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<td>Thr/Tyr kinase (MEK)</td>
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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 (rbo) [59] may play a key role in controlling the spatial activation of DER. The expression pattern of rbo is extremely dynamic, and correlates well with the domains where DER activity is required. For example, during mid-stages of oogenesis, rbo is expressed on the apical surface of the dorsal-anterior follicle cells [60**], which require DER activity for normal differentiation [32*]. Lack of rbo 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 rbo, analyzed by ectopic expression experiments, has shown that ubiquitous expression of rbo 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 rbo [26,27,31***,59]. Because spi and DER are broadly expressed during embryogenesis and because rbo 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, rbo 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.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


A genetic screen for suppressors of a weak gain-of-function torso mutation led to the isolation of more than 40 mutations. These mutations define at least seven complementation groups, among which are Sos and p21ras/Ras1.


Analysis of the role of the Drosophila Raf kinase in the R7 pathway indicates that Raf acts downstream of Ras1 and upstream of sina in sevenless signalling.


12. HART AC, KRAMER H, VAN VCTOR DL, PAHUJNAGT M, ZIPURSKY SL: Induction of Cell Fate in the Drosophila
Retina: the Bride of Sevenless Protein is Predicted to Contain a Large Extracellular Domain and Seven Transmembrane Segments. *Genes Dev* 1990, 4:1835-1847.


Results in this paper demonstrate that Tor activity is controlled by a diffusible ligand present in the perivitelline space. Analyses of *tor* mutations indicate that Tor acts as a tyrosine kinase and that gain-of-function mutations are associated with ligand-independent activation.


This paper describes the molecular characterization of the putative torso ligand encoded by *asl*. The putative secreted protein is expressed in specialized follicle cells located at the poles of the egg chamber.


This paper reports the cloning of *Spi*, a TGFα-like protein, and its cellular localization. In addition, a description of the multiple roles of *spi* during embryonic development is provided.


This paper reports the cloning of *Grk*, a TGFα-like protein, and its cellular localization. *Grk* RNAs become asymmetrically localized to the dorsal corner of the ounce during oogenesis.


This paper reports the characterization of mutations in a *Drosophila* FGF receptor homolog. The gene was called *breathless* and includes defects in tracheal-cell migration.


This paper describes the molecular characterization of *E(*sew)*2B, a gene previously identified in screens for enhancers of *sevenless*. *E(*sew)*2B encodes a protein similar to *Grb2* and *Sem-5* proteins, which contain two *SH3* and one *SH2* motifs. Evidence is provided that this protein acts upstream of *Ras* in sevenless signalling and as an adaptor protein between sevenless and *Sos*.


A *Drosophila* cDNA that encodes a protein similar to *Grb2* and *Sem-5* is shown to identify the *E(*sew)*2B locus. The authors demonstrate that the *SH2* domain of *Sos* mediates binding to activated sevenless.

In addition, the *SH3* domains mediate direct binding between the *SH3* domains and a proline-rich region at the carboxy-terminal tail of *Sos*. 

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Cell regulation


This paper demonstrates that expression of an activated form of p21ras/Ras1 in the ommatidium induces the recruitment of extra R7 photoreceptor cells.


This paper demonstrates that p21ras/Ras1 is an intrinsic component of Torso signalling. Injection of an activated form of p21ras rescues the maternal effect of torso but not of D-ras mutation. Injection of a dominant-negative form of p21ras in wild-type embryos generates a terminal class phenotype. In addition, it is shown that Ras is associated with a terminal class maternal-effect phenotype.


This paper presents evidence that Rho leads to selective activation of DER signalling pathway during vein formation.


A gain-of-function mutation, Dsor1, was isolated as a suppressor of a weak D-raf mutation. Molecular characterization of Dsor1 identified a Drosophila homologue of the tyrosine/threonine kinase MEK, suggesting that Dsor1 is an activating mutation of MEK.


This paper describes the isolation of a Drosophila non-receptor protein tyrosine phosphatase that contains two SH2 domains. Csw activity is required for proper development of the embryonic terminal lates. It is shown that csw encodes a molecule involved in Torso signalling, as maternal csw activity is necessary for expression of the dominant phenotype associated with a gain-of-function mutation.


This paper provides evidence that Rho leads to selective activation of DER in the dorsal follicle cells.


This paper provides evidence that rho leads to selective activation of DER signalling pathway during vein formation.


