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REVIEW

The Torso Pathway in *Drosophila*: Lessons on Receptor Tyrosine Kinase Signaling and Pattern Formation

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Pattern formation at the anterior and posterior termini of the *Drosophila* embryo involves intercellular communication via the Torso receptor tyrosine kinase (RTK). Recent advances in the understanding of Torso signaling has provided further support for the conservation of a signal transduction cassette downstream of RTKs. In addition, the analysis of the Torso pathway has begun to reveal general molecular mechanisms by which cells may impart patterning information to their neighbors through the use of RTKs.

I. INTRODUCTION

Developmental biologists have long sought to understand the generation of complex patterns from seemingly homogeneous structures. However, only recently has the molecular basis of pattern formation begun to reveal its secrets. Unifying concepts have begun to develop from work in a variety of developmental systems. In particular, the analysis of receptor tyrosine kinase (RTK) signaling in *Drosophila melanogaster*, *Caenorhabditis elegans*, and vertebrates has led to the identification of one such unifying concept: RTKs provide an efficient molecular mechanism by which intercellular patterning information can be transduced through a conserved signal transducing cascade.

Activation of a membrane-bound receptor by an extracellular ligand is a common mechanism utilized to transduce information between neighboring cells. Recently, years of biochemical, genetic, and molecular work have converged to demonstrate that one particular class of membrane-bound receptors functions by activating a conserved signal transduction cascade. We now know that transmembrane receptors of the protein tyrosine kinase family share not only structural homology, but also the ability to regulate the activity of similar downstream molecules (For reviews see Williams, 1992; Egan and Weinberg, 1993; Perrimon, 1993; Moodie and Wolfman, 1994). Many of these downstream effectors had been previously identified in vertebrate systems as important components in cellular transformation, while in invertebrate systems they had been isolated as mutants affecting cell fate determination and pattern formation. It is the merging of these many different lines of research that has provided us with a better understanding of the role of these signaling molecules and cell-cell communication in cell fate determination, growth, and differentiation during development. In this article we will review the contributions of one particular invertebrate RTK signaling system, that of the *Drosophila* terminal or Torso (*tor*) pathway, to our understanding of RTK signaling and pattern formation.

II. SETTING COORDINATES: THE TERMINAL SYSTEM IS ONE OF FOUR MATERNAL PATTERNING SYSTEMS

Embryogenesis is an extremely rapid process in *Drosophila*, taking only 24 hr to complete (for detailed description see Campos-Ortega and Hartenstein, 1985). Following fertilization the embryo undergoes 13 rapid rounds of nuclear division in the absence of cell division to generate a syncytial blastoderm. After the thirteenth cycle development slows to allow cellularization to be completed during the fourteenth cell cycle. This cellular partitioning converts the embryonic syncytium to a cellular blastoderm. At this point individual cells have become committed to a particular fate with respect to the anteroposterior (a/p) and dorsoventral (d/v) axes and the fate map of the larval body has been established (Fig. 1). This pattern is readily apparent in the larval cuticle, which is secreted during the last stages of embryogenesis by the underlying epidermis (Fig. 1).

Using the organization of the cuticle as an assay for body pattern classical genetic approaches were undertaken in the late 1970s and early 1980s to identify genes responsible for embryonic patterning (for reviews see St. Johnston and Nüsslein-Volhard, 1992; Chasan and Anderson, 1993; Driever, 1993; Martinez-Arias, 1993;

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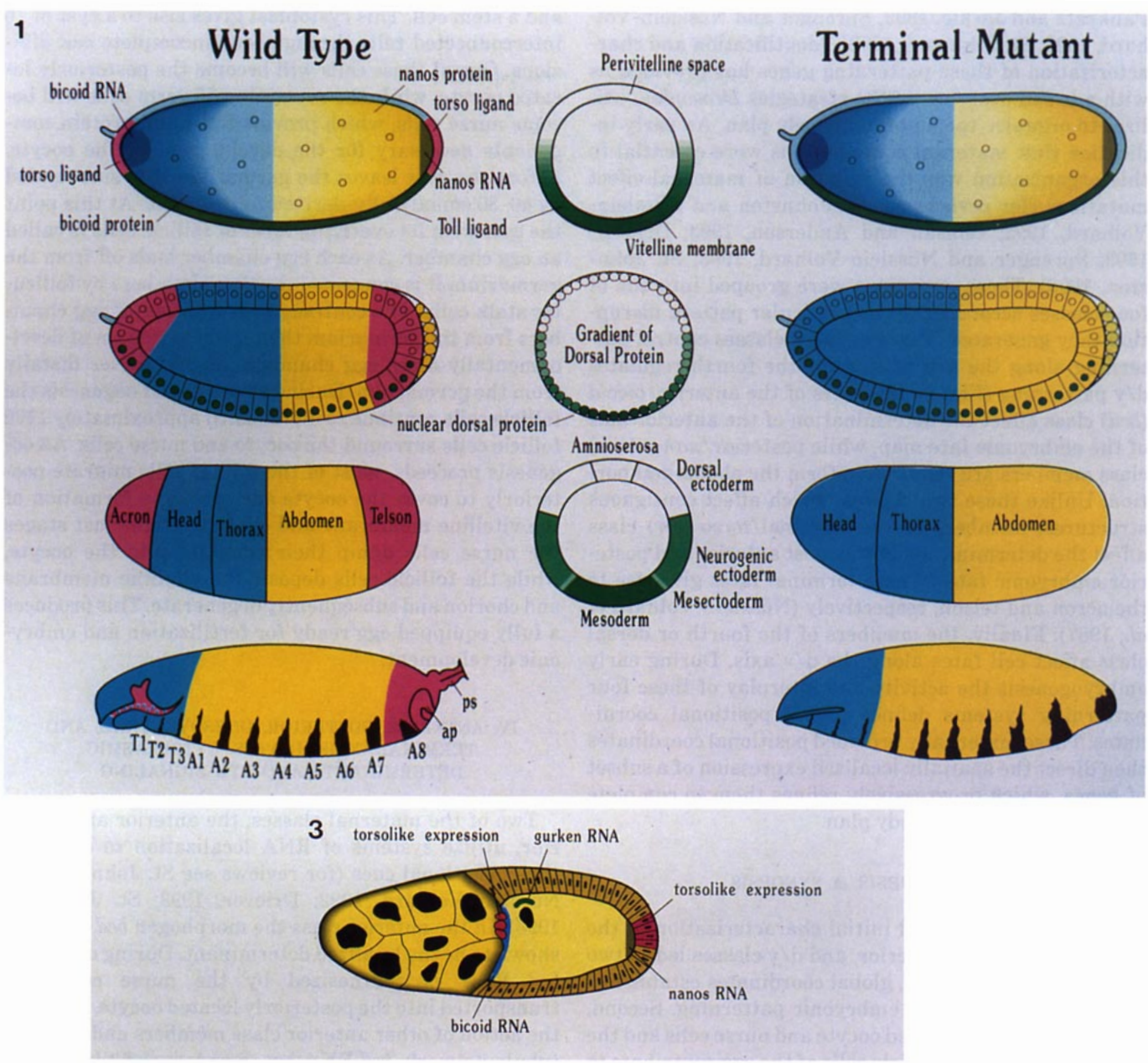

FIG. 1. Maternal patterning of the embryonic body axes. In the wild-type embryo four maternal systems organize pattern along the anterio-posterior (a/p) and dorsoventral axes (d/v). In the syncytial embryo the localized RNAs, *bicoid* (*bcd*, blue) and *nanos* (*nos*, yellow), are translated into protein gradients responsible for determining the anterior (head and thorax, blue) and posterior (abdominal, yellow) regions, respectively. At the termini Torso (*tor*) is activated by a localized ligand (red) present in the perivitelline space. This results in the activation of a signaling cascade that determines the anterior and posterior terminal structures (red)—the acron and telson, respectively. In the dorsal-ventral (d/v) axis (see cross sections), determination of the follicular epithelium via the *gurken* (*grk*)/*Drosophila* EGF receptor (*DER*) pathway affects the ventral activation of Toll ligand (green) in the perivitelline fluid. Ventral activation of Toll, as a result of this localized ligand, produces a graded nuclear distribution of *dorsal* (*dor*) protein (green) along the d/v axis and the specification of ventral structures. In terminal class mutants embryos anterior terminal structures are disrupted and posterior terminal structures are deleted. Concomitant with this loss of terminal activity is a corresponding expansion of central and subterminal patterning information (*bcd* and *nos*).

FIG. 3. Localized components of the maternal patterning systems during oogenesis. The distribution of the key localized components for each of the four maternal patterning systems is depicted in a stage 10 egg chamber. In the anterior *bcd* (blue) and posterior *nos* (yellow) systems RNAs are localized to the anterior and posterior of the developing oocyte, respectively. Terminal activity is presumably localized by the expression of *torsolike* (*tsl*, red), which is restricted to polar follicle cells. In the d/v system localization of *grk* (green) RNA to a dorsal-anterior cap over the oocyte nucleus organizes patterning information.

Pankratz and Jackle, 1993; Sprenger and Nüsslein-Volhard, 1993; St. Johnston, 1993). Identification and characterization of these patterning genes has provided us with a broad overview of the strategies *Drosophila* utilizes to organize the embryonic body plan. An early indication that maternal contributions were essential to this organization was the isolation of maternal-effect mutations (for reviews see St. Johnston and Nüsslein-Volhard, 1992; Chasan and Anderson, 1993; Driever, 1993; Sprenger and Nüsslein-Volhard, 1993; St. Johnston, 1993). These mutations were grouped into one of four classes according to the particular pattern disruption they generated. Three of these classes control patterning along the a/p axis, while the fourth regulates d/v patterning (Fig. 1). Members of the anterior/*bicoid* (*bcd*) class affect the determination of the anterior half of the embryonic fate map, while posterior/*nanos* (*nos*) class members are required to form the abdominal portion. Unlike these two classes, which affect contiguous structures, members of the terminal/*torso* (*tor*) class affect the determination of the most anterior and posterior embryonic fates. These terminal fates give rise to the acron and telson, respectively (Nüsslein-Volhard *et al.*, 1987). Finally, the members of the fourth or dorsal class affect cell fates along the d/v axis. During early embryogenesis the activity and interplay of these four patterning systems defines global positional coordinates. These maternally provided positional coordinates then direct the spatially localized expression of a subset of genes, which progressively refines them to complete the elaboration of the body plan.

III. OOGENESIS: A SYNOPSIS

The identification and initial characterization of the terminal, anterior, posterior, and d/v classes led to two major discoveries. First, global coordinates established during oogenesis direct embryonic patterning. Second, both the germline-derived oocyte and nurse cells and the somatically-derived follicle cells of the egg contribute to the organization of these global coordinates (for reviews see St. Johnston and Nüsslein-Volhard, 1992; Chasan and Anderson, 1993; Driever, 1993; Sprenger and Nüsslein-Volhard, 1993; St. Johnston, 1993). To understand how the terminal system contributes to establishing these spatial coordinates we first need to review oogenesis (Fig. 2; for a more extensive review see Spradling, 1993).

The reproductive system of *Drosophila* consists of two ovaries each of which contains 15–20 ovarioles (Fig. 2). At the apical end of an ovariole lies the germarium, which acts as a production center for egg chambers. Germline stem cells divide continuously in the germarium with each division producing a daughter cystoblast and a stem cell. This cystoblast gives rise to a cyst of 16 interconnected cells through four incomplete cell divisions. One of these cells will become the posteriorly located oocyte, while the remaining 15 germ cells will become nurse cells, which provide RNA and protein components necessary for the development of the oocyte. Before the cyst leaves the germarium it is surrounded by 60–80 somatically-derived follicle cells. At this point the cyst with its overlying layer of follicle cells is called an egg chamber. As each egg chamber buds off from the germarium it is connected to other chambers by follicular stalk cells. The continuous generation of egg chambers from the germarium then leads to a chain of developmentally older egg chambers as one moves distally from the germarium. During stages 1–6 of oogenesis the follicle cells continue to divide until approximately 1100 follicle cells surround the oocyte and nurse cells. As oogenesis proceeds, most of the follicle cells migrate posteriorly to cover the oocyte and start the formation of the vitelline membrane and chorion. In the last stages the nurse cells dump their contents into the oocyte, while the follicle cells deposit the vitelline membrane and chorion and subsequently degenerate. This produces a fully equipped egg ready for fertilization and embryonic development.

IV. ANTERIOR, POSTERIOR, DORSOVENTRAL, AND TERMINAL COORDINATES: CYTOPLASMIC DETERMINANTS AND RTK SIGNALING

Two of the maternal classes, the anterior and posterior, utilize systems of RNA localization to establish their positional cues (for reviews see St. Johnston and Nüsslein-Volhard, 1992; Driever, 1993; St. Johnston, 1993). In the anterior class the morphogen *bcd* has been shown to be the localized determinant. During oogenesis *bcd* RNA is synthesized by the nurse cells and transported into the posteriorly located oocyte. Through the action of other anterior class members and a microtubule network, *bcd* RNA becomes trapped as it enters the anterior of the oocyte, producing an anteriorly localized distribution of RNA (Fig. 3). In the posterior class *nos* has been demonstrated to be the critical localized determinant. As with *bcd*, *nos* RNA is synthesized by the nurse cell complex and transported into the oocyte. However, with the aid of other posterior class members *nos* RNA is localized to the posterior region of the oocyte (Fig. 3). Upon fertilization, translation of these localized RNAs in the embryonic syncytium allows for diffusion and results in an anterior *bcd* or posterior *nos* gradient, respectively (Fig. 1). These gradients then direct the spatial transcription of a subset of zygotic genes. This occurs either directly in the case of *bcd*, which encodes a homoedomain-containing protein or indirectly in the

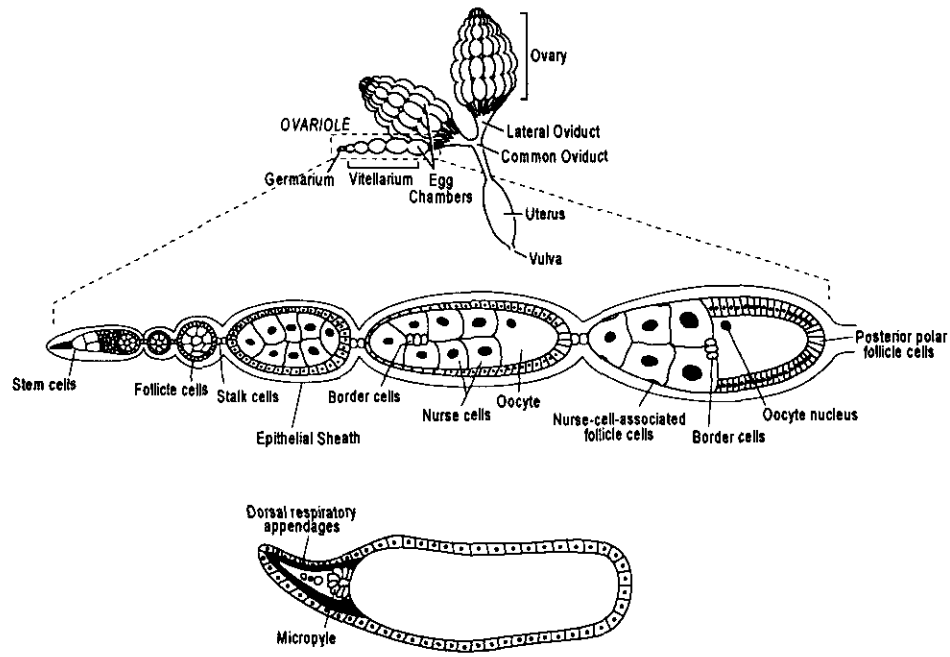

FIG. 2. Ovarian development in *Drosophila:* a synopsis. Ovarian development is schematically diagrammed with key features of oogenesis depicted. For a detailed description refer to text section III.

case of *nos*, which controls the translation of the maternal transcription factor *hunchback* (*hb*).

Unlike the anterior and posterior systems, the terminal system requires intercellular communication via a RTK to direct its positional cues (for reviews see St. Johnston and Nüsslein-Volhard, 1992; Chasan and Anderson, 1993; Sprenger and Nüsslein-Volhard, 1993). In this respect patterning along the dorsoventral axis is similar to terminal patterning—it also requires intercellular signaling via a RTK. This discovery was fueled first by the discovery that communication between the somatically-derived follicle cells and the germline-derived oocyte was crucial to the specification of terminal and d/v structures and second, by the molecular demonstration that the *tor* gene of the terminal class and the *torpedo* gene (*Drosophila* EGF receptor—DER) of the d/v class both coded for transmembrane receptors of the protein tyrosine kinase family (Schüpbach and Wieschaus, 1986; Schüpbach, 1987; Casanova and Struhl, 1989; Price *et al.*, 1989; Schejter and Shilo, 1989; Sprenger *et al.*, 1989). While these similarities exist for the two systems, it should be noted that their direction of signaling is different. In the terminal system signaling from the follicular epithelium leads to activation of the Tor RTK in the germline, and for the dorsoventral system signaling from the germline leads to activation of the DER RTK in the follicular epithelium. Over the next sections we will discuss in detail the signaling mechanisms coordinating the relay of information through the terminal class and in the final section discuss the similarities that exist between the Tor/terminal system and the DER-mediated portion of the d/v patterning system.

V. TERMINAL DEVELOPMENT: LOSS-OF-FUNCTION AND GAIN-OF-FUNCTION PHENOTYPES

a. The Loss-of-Function (LOF) Phenotype

To date nine loci that are maternally required for organizing the terminal regions of the larva have been identified through classical genetic screens and loss-of-function phenotypes. Although all of these genes are required for the specification of both the acron (defined here as including the labrum, cephalopharyngeal skeleton, and portions of the optic lobes) and the telson (defined here as all structures posterior to and including abdominal segment eight: posterior spiracles, filzkorper, Malphigian tubules, and derivatives of A9, 10, 11) their activities are required in different tissues. Eight of these genes are required in the germline, while the activity of the ninth, *torsolike* (*tsl*), is required in the somatically derived follicle cells. However, in addition to the activity of the terminal class genes, the specification of the acron also requires the activity of the anterior morphogen *bcd*. In the absence of *bcd*, terminal class activity leads to a duplicated telson being formed anteriorly in place of the lost acron.

At the cuticular level loss of the maternal activity of

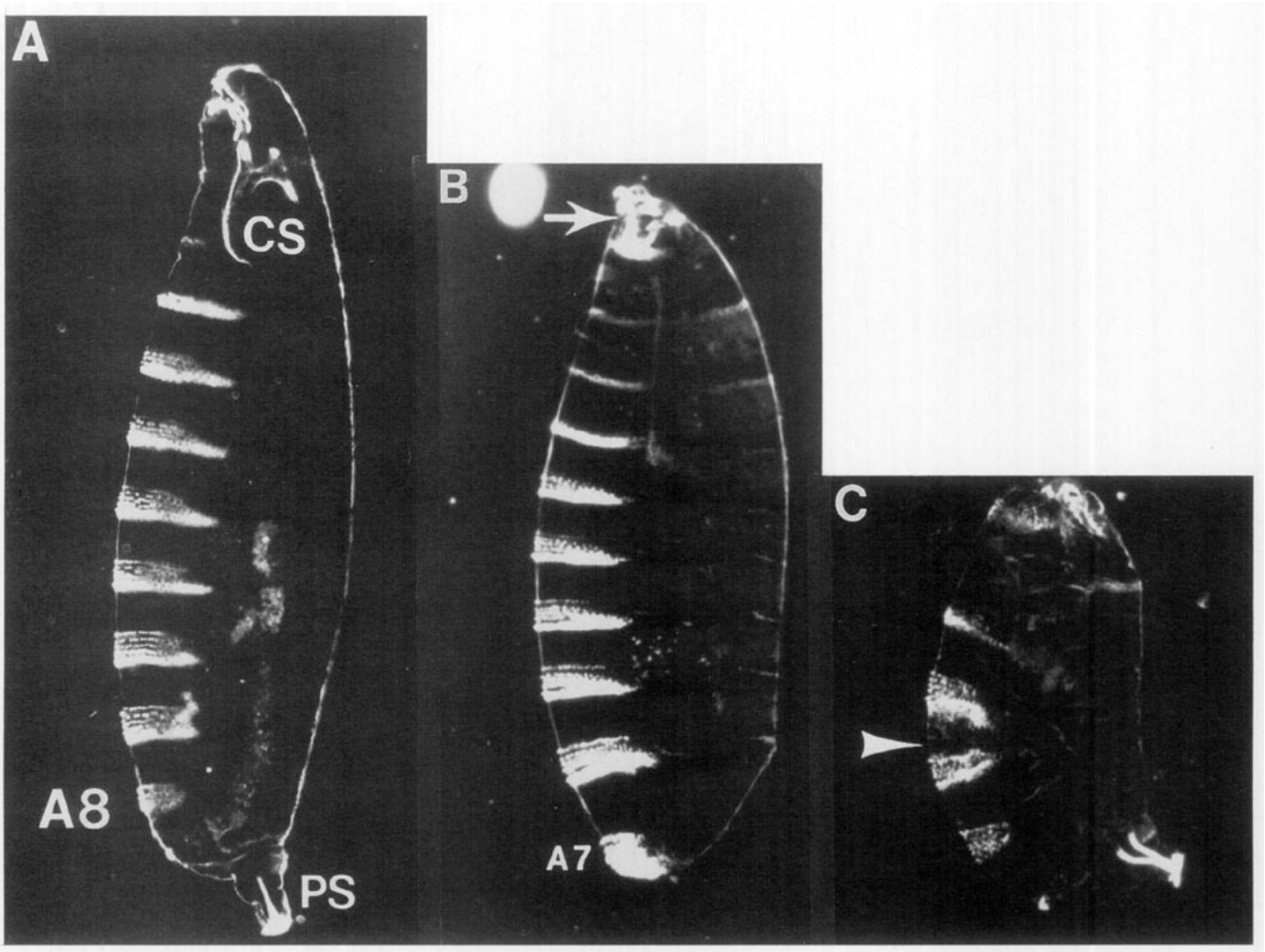

FIG. 4. Embryonic terminal class phenotypes. Dark-field photographs of the embryonic cuticular elements present in a wild-type (A), tor^{LOF} (B), and tor^{GOF} (C) are shown. (A). In addition to the three thoracic and eight abdominal segments marked by the presence of ventral denticle belts, the anterior cephalopharyngeal skeleton (cs) and posterior posterior spiracles (ps) terminal structures are marked. (B). In embryos lacking terminal class activity the cephalopharyngeal skeleton is reduced and all structures posterior to abdominal segment 7 are deleted. (C). In embryos with ectopic terminal class activity abdominal segmentation is repressed (arrowhead). Courtesy of X. Lu.

eight of the terminal class genes leads to a qualitatively similar phenotype: loss of the acron and telson (Figs. 1 and 4). The only exception to this phenotypic classification is the gene *corkscrew* (*csw*) since *csw* mutants affect only a subset of these structures (Perkins *et al.*, 1992).

Analysis of the terminal class phenotype at the cellular level indicates that the failure to specify the terminal fates does not lead to cell death. Instead, loss of terminal class activity leads to a respecification of the terminal regions (Klinger *et al.*, 1988; Strecker *et al.*, 1989). This is accomplished through the derepression and concomitant expansion of central and subterminal patterning information into the terminal regions. This respecification of the terminal anlagen of the larva can be visualized through the expression of zygotic molecular markers that distinguish regions of the fate map, such as the terminal gap genes *tailless* (*tll*) and *huckebein* (*hkb*), the subterminal gap gene *giant* (*gt*), and the pair-rule gene *fushi tarazu* (*ftz*) (Fig. 5; Klinger *et al.*, 1988; Casanova and Struhl, 1989; Strecker *et al.*, 1989; Weigel *et al.*, 1990; Bronner and Jackle, 1991; Eldon and Pirrota, 1991; Kraut and Levine, 1991; Pignoni *et al.*, 1992). The early zygotic expression of *tll* and *hkb* mark terminal regions of the anlagen, while the expression of other gap genes marks the central and subterminal regions of the anlagen (for reviews see Jurgens and Hartenstein, 1993; Pankratz and Jackle, 1993). In response to decreased terminal class activity *tll* and *hkb* expression is reduced or lost at the termini with a coincident shift of central and subterminal information into these regions.

b. The Gain-of-Function (GOF) Phenotype: Ordering the Pathway

An important contribution to our understanding of the terminal class patterning system was the discovery

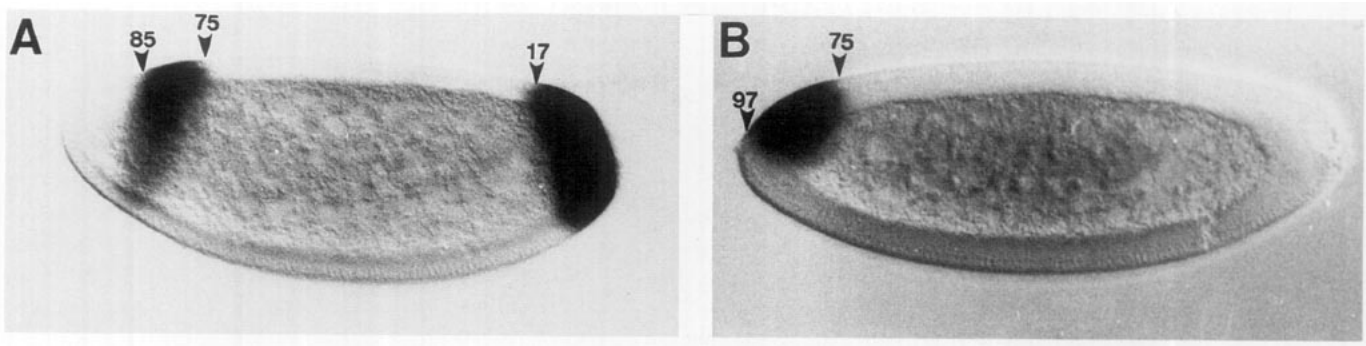

FIG. 5. Loss of terminal class activity leads to a loss of cell fates. The *in situ* hybridization pattern of *tailless* (*tll*) of a wild-type (A) and tor^{LOF} (B) embryo are shown. (A). The anterior and posterior expression domains of *tll* in a wild-type embryo at cellular blastoderm are indicated. (B). In a tor^{LOF} embryo *tll* expression is disrupted at the anterior and deleted at the posterior. Courtesy of X. Lu.

of maternal mutations producing the "spliced" phenotype (Klinger *et al.*, 1988; Schüpbach and Wieschaus, 1989; Szabad *et al.*, 1989). These mutations do not affect the development of the embryonic termini, but instead suppress the pattern of central and subterminal cuticular elements: the segmented regions of the head, thorax, and abdomen. In spliced embryos varying degrees of these segmented regions are suppressed and respecified as terminal pattern elements (Fig. 4; Klinger *et al.*, 1988; Strecker *et al.*, 1989; Szabad *et al.*, 1989). At the molecular level this shift in the fate map can be visualized by expanded *tll* expression and the concurrent suppression of segmentation gene expression (Klinger *et al.*, 1988; Strecker *et al.*, 1989; Bronner and Jackle, 1991; Pignoni *et al.*, 1992). All three of the mutations that produce the embryonic spliced phenotype have been shown to be GOF alleles of the terminal gene *torso* (tor^{RL3}, tor^{Y9}, and $tor^{4021} = tor^{GOF}$) (Klinger *et al.*, 1988; Szabad *et al.*, 1989).

As described above tor^{GOF} mutations result in a phenotype that is reciprocal to the terminal class LOF phenotype. In tor^{GOF} mutations terminal fates are expanded, while in LOF mutations terminal fates are reduced or absent. This observation prompts a simple question: Is the expansion of terminal pattern elements in the tor^{GOF} phenotype dependent upon the activity of other maternal terminal class genes? By combining a terminal class LOF mutation with a tor^{GOF} mutation one can determine if the expanded terminal elements in tor^{GOF} embryos still require the activity of that particular terminal class gene and order that gene with respect to *tor* activity. These double mutant analyses established a pathway for terminal development with *tor* as the central component. Expansion of terminal elements in the tor^{GOF} phenotype does not require the activity of the terminal genes *fs(1)Nasrat (fs(1)N), fs(1)polehole (fs(1)ph), torsolike (tsl),* and *trunk (trk),* but does require the activity of the *Drosophila raf (D-raf* or *l(1)polehole)* gene, *csw,* and *Drosophila suppressor of raf-1* (*Dsor-1* or *DMEK-1*) (Casanova and Struhl, 1989; Ambrosio *et al.*, 1989b; Stevens *et al.*, 1990; Perkins *et al.*, 1992; Tsuda *et al.*, 1993).

In addition to providing a means to order terminal class members with respect to *tor*, the GOF alleles also suggested that *tor* activity is normally limited to the terminal regions of the embryo. However, unlike the localized cytoplasmic activities demonstrated for the maternal anterior and posterior classes rescuing activity for *tor* via cytoplasmic transplantation was found in all regions of the early embryo (Klinger *et al.*, 1988; Sugiyama and Okada, 1990).

VI. MOLECULAR ANALYSIS OF TERMINAL DEVELOPMENT

a. *The Central Element—Torso: A Receptor Tyrosine Kinase (RTK)*

While the isolation of LOF and GOF alleles suggested that *tor,* and thus terminal class, activity is restricted to the termini, the inability to obtain a localized activity for *tor* through cytoplasmic transplantation raised the following question: How do the members of the terminal class generate an activity that is localized to the terminal regions of the embryo? The subsequent molecular isolation and characterization of *tor* provided the first important clue toward answering this question and confirmed the placement of *tor* at a central point in the terminal pathway. Sequence analysis of *tor* indicated that it encoded a putative transmembrane member of the RTK family (Casanova and Struhl, 1989; Sprenger *et al.*, 1989). Structurally, the *tor* protein (Tor) consists of an N-terminal extracellular region, a single membrane-spanning region, and a C-terminal cytoplasmic region containing the kinase domain. Interestingly, while the extracellular region of Tor exhibits no similarity to other RTK's, the structure of its kinase domain identifies it as a member of a particular class of RTK's. All members of this class contain a stretch of hydrophilic amino acids that interrupts the cytoplasmic kinase do-

main, but does not abolish its function (for review see Ullrich and Schlessinger, 1990).

The biochemical and histochemical characterization of Tor has provided further support for its classification as a RTK. Sprenger and others (1992, 1993) have begun a detailed analysis of the kinase activity of Tor (Sprenger and Nüsslein-Volhard, 1992; Sprenger and Nüsslein-Volhard, 1993; Sprenger *et al.*, 1993). Activation of most RTK's involves ligand-induced dimerization and intermolecular autophosphorylation of the dimerized receptors (for review see Ullrich and Schlessinger, 1990). Thus, as expected, Tor is capable of tyrosine autophosphorylation. Furthermore, mutations in the Tor kinase domain that impair or abolish kinase activity lead to loss of terminal fates (LOF mutations). In contrast, GOF mutations that result in constitutive kinase activity (which is ligand-independent) and produce excess terminal fates all lie in the extracellular region of Tor. This provided a molecular explanation for the reciprocal phenotypic effects of tor^{LOF} and tor^{GOF} mutations.

Histochemically, Tor is uniformally distributed along the cell surface of the embryonic syncytium (Casanova and Struhl, 1989; Stevens and Nüsslein-Volhard, 1991). This is consistent with Tor acting as a *receptor* tyrosine kinase. Moreover this provided an explanation for the inability to recover localized cytoplasmic Tor activity. Tor is membrane-bound and its expression is not restricted to the terminal regions of the embryo. This, coupled with the identification of Tor as a RTK, led to the following scenario for the specification of terminal fates. A localized extracellular signal produced through the action of *tsl*, *trk*, *fs(1)N*, and *fs(1)ph* results in the activation of Tor in the terminal regions, thereby initiating a cellular signaling cascade that leads to the specification of terminal fates.

b. *Upstream of Torso: From the Soma to the Germline*

1. Torsolike—The Localized Component. While the activation of RTK's usually requires binding of a ligand to the receptor, the extracellular domain of Tor provided no insight as to the nature of such potential ligands. Genetically however, such a molecule would have to function upstream of the receptor. The epistasis experiments described in section Vb indicated that four of the terminal class genes: *tsl*, *trk*, *fs(1)N*, and *fs(1)ph* act upstream of *tor*. The activity of three of these: *trk*, *fs(1)N*, and *fs(1)ph*, is required in the germline, while the fourth, *tsl*, is required in the somatically derived follicle cells (Perrimon *et al.*, 1986; Schüpbach and Wieschaus, 1986; Stevens *et al.*, 1990). These cells, which surround the oocyte and deposit the vitelline membrane and chorion during oogenesis, contain polar subpopulations located at the anterior and posterior of the developing oocyte (Spradling, 1993). These polar follicle cells provide a potential source for a ligand localized to the terminal regions of the egg.

In an elegant approach, Stevens *et al.* (1990) demonstrated that the activity of *tsl* is indeed localized to these polar follicle cells (Stevens *et al.*, 1990). When marked tsl^- clones were generated in the follicular epithelium seven embryos with posterior terminal defects were recovered. Of these seven embryos three were derived from egg chambers in which only posterior polar follicle cells were mutant for *tsl*. This demonstrated that *tsl* activity is required only in the polar follicle cells for the specification of terminal fates. It also indicated that the specification of terminal fates occurs independently at the two ends of the embryo since embryos with only posterior defects were recovered. Based on these results then, *tsl* satisfies all of the genetic requirements for the putative ligand of Tor.

The recent isolation and molecular characterization of *tsl* has indicated that it has all of the molecular attributes predicted for the Tor ligand (Savant-Bhonsale and Montell, 1993; Martin *et al.*, 1994). As expected from the mosaic analysis, *tsl* expression is restricted during oogenesis to polar follicle cells. At the anterior, *tsl* is expressed in the border cells. This group of 6–10 cells originates at the anterior of the egg chamber and during the latter stages of oogenesis (9/10) migrate through the nurse cells, where they come to lie at the anterior of the oocyte, directly opposed to the oocyte nucleus. In the posterior region, *tsl* is also expressed in polar follicle cells juxtaposed to the posterior pole of the oocyte. Second, consistent with the requirement for a secreted molecule, the Tsl protein contains a putative signal sequence and no transmembrane domain. Tsl also appears to have no significant similarity to any known proteins. Third, Tsl protein is localized to the anterior and posterior termini of the syncytial embryo (Martin *et al.*, 1994). Finally, the ectopic, unrestricted expression of *tsl* during oogenesis leads to an expansion of terminal fates, thereby producing a cuticular phenotype similar to the tor^{GOF} phenotype. This GOF phenotype is most likely the result of the uniform activation of Tor throughout the embryo as a consequence of the ubiquitous expression of *tsl*. If this presumption is true, this experiment demonstrates that all of the components necessary for the activation of the terminal pathway, with the exception of *tsl*, are homogeneously distributed throughout the embryo.

2. trunk, fs(1)Nasrat, and fs(1)Polehole—In Search of a Function. Given the localization and molecular identity of *tsl* and the RTK nature of *tor*, what roles do *trk*, *fs(1)N*, and *fs(1)ph* have in regulating terminal activity? Genetically, these three genes are all required in the

germline and function upstream of *tor* (Perrimon *et al.*, 1986; Schüpbach and Wieschaus, 1986; Klinger *et al.*, 1988; Stevens *et al.*, 1990). Furthermore, they are not involved in regulating the expression of *tor* (Casanova and Struhl, 1989; Stevens and Nüsslein-Volhard, 1991). Most likely, *trk*, *fs(1)N*, and *fs(1)ph* are involved in regulating the expression, localization, or activity of Tsl. Evidence for such potential roles comes from two lines of study. The first involves the timing of Tor activation and the diffusible nature of the Tor ligand, while the second involves the pleiotropic nature of *fs(1)N*, and *fs(1)ph* phenotypes.

In order to address the temporal aspect of Tor activation Sprenger and Nüsslein-Volhard (1992) made the following observations. First, injection of *tor* mRNA (encoding a wild-type Tor protein) into embryos derived from tor^{LOF} females is capable of rescuing the terminal defects associated with tor^{LOF} mutations. They demonstrated that these terminal defects are capable of being rescued if the injection of *tor* mRNA is performed prior to cellular blastoderm. This suggests that Tor expression is required during the first two hours of embryogenesis. Consistent with this Tor is expressed during the first four hours of development (Casanova and Struhl, 1989; Stevens and Nüsslein-Volhard, 1991; Sprenger and Nüsslein-Volhard, 1992). Furthermore, the tyrosine phosphorylation, and thus activity, of Tor peaks from 1–2 hr of development (Sprenger and Nüsslein-Volhard, 1993; Sprenger *et al.*, 1993). If activation of Tor occurs in response to ligand binding, then these experiments indicate that the Tor ligand must be present during the first 2 hr of embryogenesis. Moreover, in the absence of Tor expression at the termini, this ligand is capable of diffusing throughout the perivitelline space during embryogenesis (Sprenger and Nüsslein-Volhard, 1992; Casanova and Struhl, 1993). If Tor expression is restricted to only the central region of the embryo, the ligand diffuses to these central regions and activates Tor here. This leads to a suppression of central fates and respecification as terminal fates, reminiscent of the tor^{GOF} alleles (Sprenger and Nüsslein-Volhard, 1992; Casanova and Struhl, 1993).

All of these results indicate that the localization of terminal activity occurs in a highly regulated temporal and spatial fashion. In the first step of this model Tsl is secreted into the perivitelline space in a localized fashion by the polar follicle cells during oogenesis. At this stage, after the deposition of the vitelline membrane and chorion and the degeneration of the follicular epithelium, how is the polar localization of Tsl maintained until Tor expression commences during embryogenesis? Failure to do so would result in the diffusion of Tsl throughout the perivitelline space and subsequent uniform activation of Tor. Therefore, Tsl apparently remains in polar locations, perhaps bound to the vitelline membrane, until the first hour of embryogenesis when Tor is expressed. After Tor expression commences Tsl is released from its terminal locations. At this stage diffusion from its polar locations coupled with an excess of Tor receptor ensures that Tor activation occurs only at the termini. Such a mechanism provides ample opportunities for the function of *trk*, *fs(1)N*, and *fs(1)ph* that are consistent with their genetic attributes.

Given that interactions between the germline and follicular epithelium have been observed for other pathways, it is possible that *trk*, *fs(1)N*, or *fs(1)ph* functions in specifying the fate of the polar follicular cells. In this scenario loss of *trk*, *fs(1)N*, and *fs(1)ph* activity would lead to misspecification of polar cells and the absence of *tsl* expression. For *fs(1)N* and *fs(1)ph*, however, their pleiotropic effects on the egg suggest an alternative scenario. While some alleles affect only terminal development, most mutations in both of these genes result in collapsed, flaccid eggs indicating a role in the ultrastructural architecture of the egg (Degelmann *et al.*, 1990). One aspect of this phenotype is the abnormal formation of the vitelline membrane. The vitelline membrane of wild-type eggs apparently ensures against osmotic imbalances; removal of the chorion of wild-type eggs has no effect, while removal of the chorion of *fs(1)N* and *fs(1)ph* mutant eggs (leaving only a vitelline membrane) results in a bursting of the embryo. These additional phenotypic effects are perhaps more consistent with a function for *fs(1)N* or *fs(1)ph* in the construction of the vitelline membrane, where they may act to tether, stabilize, or release Tsl from the polar regions of the vitelline membrane.

Unlike *fs(1)N* and *fs(1)ph*, however, *trk* mutations do not appear to have ultrastructural effects. Without knowing the molecular function of *trk* and in the absence of direct biochemical evidence that Tsl binds to Tor, *trk* could function in a variety of ways. For instance, *trk* could encode a Tor ligand that is ubiquitously expressed during embryogenesis, but which requires modification by Tsl at the polar regions to properly activate Tor. Interestingly, *trk* does appear to encode a secreted molecule (cited in Casanova and Struhl, 1993). Regardless, the molecular identification of *trk*, *fs(1)N*, and *fs(1)ph* will continue to elucidate the mechanisms underlying the extracellular regulation of a RTK.

Finally, the availability of antibody to Tsl coupled with the polar localization of Tsl in syncytial embryos provides a straightforward way to test the function of *trk*, *fs(1)N*, and *fs(1)ph* in localizing terminal activity and determine if Tsl is the Tor ligand. For example, what is the localization of Tsl in mutants for *trk*, *fs(1)N*, *fs(1)ph*, and *tor*?

c. *Downstream of Torso: From the Membrane to the Nucleus*

The activation of Tor leads to the nucleus via a pathway that is apparently common to many different RTK's in a variety of eukaryotic systems (for reviews see Perrimon, 1993; Moodie and Wolfman, 1994). Central to this pathway in mammalian systems is the activity of *ras*, the prototypical member of a family of small guanine-nucleotide-binding proteins (for reviews see Downward, 1992; Pelech, 1993). As one might predict for a protein involved in the early phases of signal transduction Ras is localized to the plasma membrane. A fatty acid chain added to its carboxy terminus links Ras to the membrane, where its activity is regulated by guanine nucleotide binding. Although able to bind GDP and GTP with similar affinities Ras family members have intrinsic GTPase activity. Ras is inactive when bound to GDP. Conversely, in the GTP-bound state Ras is active and stimulates a kinase cascade that eventually leads to the nucleus. In *Drosophila*, *Ras1*, a homolog of mammalian *ras*, apparently acts in a similar fashion (Simon *et al.*, 1991). Here Ras1 is required for the transmission of activity from the Sev and DER RTK's (Simon *et al.*, 1991; Dickson and Hafen, 1993). In addition, biochemical and genetic evidence suggest that Ras1 may also play a role in transmitting the activity of the Tor RTK (Doyle and Bishop, 1993; Lu *et al.*, 1993).

As described earlier, the ubiquitous activation of Tor results in an expansion of terminal information, thereby giving rise to the spliced phenotype. Similarly, ectopic expression of a constitutively activated form of Ras1 also results in an expansion of terminal information and a spliced-like phenotype (Lu *et al.*, 1993). In addition, expression of this activated form of Ras1 is capable of restoring the terminal structures associated with a loss in Tor activity (Lu *et al.*, 1993). Conversely, expression of a dominant negative form of Ras1 inhibits terminal activity. Finally, reduction of Ras1 activity partially suppresses the spliced phenotype generated by the tor^{GOF} mutations (Doyle and Bishop, 1993). These results suggest that Ras1 activity is important in the transduction of terminal information. However, experiments utilizing activated or inhibitory situations could also be interpreted as overriding or competing with an endogenous (non-Ras1) pathway. Thus, it remains to be determined if the sole removal of Ras1 activity from the maternal germline results in failure to transduce terminal information. Until this question has been answered, alternative (non-Ras1) mechanisms for the transduction of Tor activity cannot be dismissed.

While all the players in the RTK-Ras pathway are still being identified, three of these signaling molecules, Son-of-sevenless (Sos), Draf, and *Drosophila* MEK1, have been genetically shown to be critical for the transmission of terminal activity (Perrimon *et al.*, 1985; Nishida *et al.*, 1988; Ambrosio *et al.*, 1989a,b; Doyle and Bishop, 1993; Lu *et al.*, 1993, 1994; Tsuda *et al.*, 1993). Like *tor*, reducing the activity of any one of these three loci in the maternal germline results in the loss of embryonic terminal fates and gives rise to a terminal class LOF phenotype.

1. Sos: an activator of Ras. If *Ras1* is a critical element in RTK signaling in *Drosophila* what regulates its state of nucleotide binding? One important clue to answering this question came from the discovery of *Sos*. *Sos* was initially identified on the basis of its positive role downstream of the Sev RTK in the specification of the R7 photoreceptor (Rogge *et al.*, 1991; Simon *et al.*, 1991). More recently, it has been shown that a reduction in *Sos* activity in the maternal germline results in a phenotype similar to that seen with partial LOF mutations in the Tor RTK (Lu *et al.*, 1993). How does Sos function in signaling from RTK's, like Tor? Sos appears to function by regulating the nucleotide-binding state of Ras.

Information concerning the function of Sos came initially from its sequence similarity to the yeast CDC25 and SDC25 guanine-nucleotide-exchange proteins (Simon *et al.*, 1991; Bonfini *et al.*, 1992). These regulators of Ras function positively by converting the inactive GDP-bound form of Ras to the active GTP-bound form (Jones *et al.*, 1991). By analogy, in the *Drosophila* terminal pathway, where Ras1 is the putative target of Tor activity, Sos would regulate Ras1 activity by increasing the proportion of GTP-bound Ras1 molecules in response to receptor activation. Consistent with this Sos regulates Ras1 activity in the *Drosophila* Sev RTK pathway and more recently has been shown to physically associate with Ras1 (Olivier *et al.*, 1993; Simon *et al.*, 1993). In contrast to these positive effects of Sos, negative regulators of Ras activity have also been identified (for review see Bollag and McCormick, 1991; Downward, 1992). These proteins enhance the GTPase activity of Ras, converting it to the GDP-bound or inactive state. While such GTPase-activating proteins (GAPs) have been shown to function downstream of some RTKs in the regulation of Ras, the role for the *Drosophila* Gap1 protein in the transduction of terminal information via Tor is unclear (Chou *et al.*, 1994).

While the analysis of Sos provided some insight, its link to Tor was still unknown. This link may lie with a molecule, termed *downstream of receptor kinases (drk)* that has been identified in *Drosophila*, as well as in other eukaryotic systems (GRB2 in humans, Sem-5 in *C. elegans*) (Clark *et al.*, 1992; Lowenstein *et al.*, 1992; Olivier *et al.*, 1993; Simon *et al.*, 1993). Drk contains two "src homology 3" (SH3) domains, separated by a single "src homology 2" (SH2) domain (for review see Pawson and

Schlessinger, 1992). With the knowledge that SH2 domains can bind directly to phosphotyrosine residues and that Drk binds to Sos via its SH3 domains, this structure suggested a mechanism by which Drk serves to link an activated RTK and Sos (for review see Pawson and Schlessinger, 1992). Upon activation RTK's undergo intermolecular autophosphorylation on tyrosine residues thereby providing a docking site for Drk. Because the SH3 domains of Drk are bound to a proline-rich region of Sos this also brings Sos into the vicinity of the activated receptor. While Drk appears to fulfill this role in the Sev RTK pathway its role in terminal development has yet to be fully clarified (Doyle and Bishop, 1993; Olivier *et al.*, 1993; Simon *et al.*, 1993). However, in support of a role for Drk in Tor signaling, reduction of Drk dosage partially suppresses the tor^{GOF} phenotype (Doyle and Bishop, 1993).

2. Draf: first in a cytoplasmic kinase cascade. Activation of Ras results in the stimulation of a kinase cascade that appears to initiate with the serine/threonine kinase Raf-1. One of the first members of the Tor pathway to be characterized at the genetic and molecular level was *Draf*, the *Drosophila* homolog of mammalian Raf-1 (Perrimon *et al.*, 1985; Nishida *et al.*, 1988; Ambrosio *et al.*, 1989a,b; Melnick *et al.*, 1993; Sprenger *et al.*, 1993).

Insight into the function of Raf-1/Draf in RTK-Ras signaling has come from many different areas, including its molecular structure. In addition to its C-terminal kinase domain Raf contains two other domains located at the N-terminus: CR1 and CR2, at least one of which, CR1, is important for signaling RTK-Ras activity. While its kinase domain is crucial for transmitting signaling activity, Raf's physical and regulatory link to RTK-Ras signaling appears to lie in CR1 (Melnick *et al.*, 1993; Vojtek *et al.*, 1993; Warne *et al.*, 1993; Zhang *et al.*, 1993; Brand and Perrimon, 1994). While deletion of the N-terminus (including CR1) results in constitutive Raf kinase activity, overexpression of the N-terminus (including CR1) acts in an inhibitory fashion (Bruder *et al.*, 1992). These results suggest that the N-terminus of Raf has an inhibitory effect on its kinase activity. Recently physical interactions between Ras and the N-terminus of Raf-1 (specifically CR1) have been documented (Vojtek *et al.*, 1993; Warne *et al.*, 1993; Zhang *et al.*, 1993). Thus, this interaction provides one possible mechanism of linking the activity of a RTK to the kinase activity of Raf-1/Draf. Consistent with the importance of this domain to biological activity a single amino acid change (Arg^{217} to leucine) in CR1 of Draf results in impaired Draf activity (perhaps by lowering the affinity of Draf for Ras1?) (Melnick *et al.*, 1993).

How does the activation of Draf by Ras continue the transduction of the information from Tor? With the knowledge that the Draf kinase domain is essential to its activity, one would predict that phosphorylation of unique substrates by Draf would continue this signaling. Utilizing the $Draf^{C110}$ mutation to screen for genetic suppressors of impaired Draf activity led to the identification of one such putative substrate of Draf: Dsor-1/DMEK-1 (Tsuda *et al.*, 1993; Lu *et al.*, 1994).

3. DMEK-1: linking Tor to the MAP kinase cascade. As mentioned above *DMEK-1* was first identified as a suppressor of the weak *Draf* allele C110 (Tsuda *et al.*, 1993; Lu *et al.*, 1994). A mutation in DMEK-1 causing partially constitutive activity was isolated on the basis of its ability to suppress the phenotypic effects associated with reduced Draf activity (Tsuda *et al.*, 1993; Lu *et al.*, 1994). In the Tor pathway this led to a significant restoration of terminal fates in embryos derived from maternal germlines with reduced Draf activity. This indicated that *DMEK-1* is capable of functioning downstream of Draf in the Tor pathway. However, proof that *DMEK-1* is required for transducing Tor activity was obtained with mutations that reduced DMEK-1 activity (Tsuda *et al.*, 1993; Lu *et al.*, 1994). As with *Tor*, *Sos*, and *Draf*, reducing the activity of DMEK-1 in the maternal germline results in loss of embryonic terminal fates and gives rise to a terminal class LOF phenotype (Tsuda *et al.*, 1993; Lu *et al.*, 1994).

Providing a nice link to mammalian studies on signal transduction the molecular analysis of DMEK-1 revealed significant sequence similarity to the mammalian MAP kinase-Erk kinase (MEK1) (Tsuda *et al.*, 1993). In response to various extracellular stimuli MEK1 acts as a tyrosine/threonine kinase capable of phosphorylating and activating MAP kinases (for review see Pelech, 1993). However, the order of events with respect to Raf-1, MEK1, and MAP kinases was not entirely clear from mammalian studies. Reports that MAP kinases phosphorylate and activate Raf-1, as well as of Raf-1 phosphorylating and activating the MAP kinase activator MEK1, have been published (Anderson *et al.*, 1991; Howe *et al.*, 1992; Kyriakis *et al.*, 1992; Lee *et al.*, 1992). The identification of DMEK1 as a suppressor of Draf strongly suggests that MEK1 acts downstream of Raf-1 and is consistent with the order of activation being Raf-1, MEK1, MAP kinase. However, epistasis experiments involving dominant-activated forms of these kinases must be interpreted with caution as these proteins can be subject to input from other pathways as well (for review see Pelech, 1993). With this caveat, the identification of DMEK1 as a member of the Tor pathway strongly suggests that the next link in the pathway would be a MAP kinase.

Interestingly, a *Drosophila* homolog of MAP kinase has been isolated at the *rolled* locus and it is capable of supplying terminal activity (Biggs and Zipursky, 1992; Biggs *et al.*, 1994; Brunner *et al.*, 1994). Dominant muta-

tions resulting in a constitutively activated form of *rolled* (DMAPK) result in a spliced phenotype similar to that seen with GOF mutations in Tor (Brunner *et al.*, 1994). Further characterization of the *rolled* locus should continue to shed light on its involvement in Tor signaling.

At this point signaling via Tor and other RTK's reaches an impasse: genes whose transcription is regulated by RTK activation have been identified, but in most cases the molecules that respond to RTK-Ras-etc. signals to direct these transcriptional changes have not (Fig. 6). To date, the transcriptional molecule(s) responsible for regulating the expression of the zygotic terminal class targets, like *tll* and *hkb*, in response to terminal activity have proven refractory to genetic analysis. As the ectopic activity of the terminal class results in the expression of these zygotic targets in more central regions, this putative transcription factor(s), often referred to as gene Y, is likely to be expressed uniformally during early embryogenesis. In more simple models, its transcriptional activity would then be dictated by post-translational modifications induced by the terminal class. The continued combination of biochemical, genetic, and molecular approaches to RTK signaling should continue to lead us in the search for these factors.

4. csw: a nonreceptor protein-tyrosine phosphatase. Finally, there is one member of the terminal class that appears to occupy a unique niche, *csw*. Unlike all other currently identified terminal class members, loss of maternal *csw* activity results in only a partial terminal class LOF phenotype—at the anterior tip, the structure of the cephalopharyngeal skeleton and dorsal bridge are disrupted, while at the posterior tip the posterior midgut and the Malphighian tubules are affected (Perkins *et al.*, 1992). However, loss of maternal *csw* activity does not affect the filzkorper or anal tuft.

Molecularly, the identification of *csw* as a putative nonreceptor protein-tyrosine phosphatase also supports its unique placement among terminal class members (Perkins *et al.*, 1992). In addition to the phosphatase domain Csw also contains two SH2 domains, indicating that like Drk, it also is capable of binding phosphotyrosine residues. In support of this a mammalian homolog of *csw*, called PTP1D (also known as SH-PTP2 or Syp) and PTP1C have been shown to bind to activated RTKs on phosphotyrosine residues and become phosphorylated on tyrosine residues (Shen *et al.*, 1991; Freeman *et al.*, 1992; Feng *et al.*, 1993; Vogel *et al.*, 1993). In addition, *csw* mutants suppress the tor^{GOF} phenotype indicating that Csw lies downstream of Tor. In spite of this and the molecular information on *csw*, the nature of its role in transducing information from Tor (or other RTK's) is still unclear.

VII. UNRESOLVED/FUTURE ISSUES

While signaling through RTKs has been under intense scrutiny recently, there are a number of questions whose answers have remained elusive. For example, do RTK's act as molecular on/off switches, or are they capable of more precise refinements in transcriptional control? What is their role in pattern formation—how does their activation generate diverse cell fates and contribute to organizing tissues? Is the concentration and or location of the receptor critical to patterning? In this last section we discuss some analogies between the Tor and DER (d/v) RTK pathways that have begun to address these issues.

a. Localizing RTK Patterning Activity: Ligand Trapping

As we mentioned earlier the activation of Tor and DER requires communication between the germline-derived oocyte and its associated follicular epithelium. In addition, these two RTK's (as is the case with other RTK's) appear to utilize many of the same downstream signaling components, such as Ras1, Draf, DMEK1, and Csw (Brand and Perrimon, 1994; Berg and Schnorr, personal communication; L. Perkins, J. C. Hsu, X. Lu, J. B. Duffy, and N. Perrimon, unpublished). In the case of Tor, RTK/patterning activity is localized to the terminal regions of the embryo by the expression of *tsl* in polar populations of follicle cells. In addition, this also requires the germline-dependent action of *trk*, *fs(1)N*, and *fs(1)ph*. In d/v patterning, DER activity is localized to the dorsal-anterior region of the follicular epithelium by the germline-dependent action of at least six loci: *gurken (grk)*, *fs(1)K10*, *squid*, *cornichon*, *cappucino*, and *spire*. (Wieschaus *et al.*, 1978; Manseau and Schüpbach, 1989; Schüpbach and Wieschaus, 1989; Kelly, 1993). In response to DER activation these follicle cells adopt a dorsal fate and give rise to dorsally located chorionic structures. In the absence of DER activity these follicular cells fail to be specified as dorsal and instead adopt the default or ventral fate, thereby giving rise to a ventralized egg lacking dorsal structures.

Grk appears to be the ligand that localizes DER RTK activity (Neumann-Silberberg and Schüpbach, 1993). *grk* encodes a dorsal-anterior localized RNA that codes for a transforming growth factor-α (TGF-α)-like molecule (Neumann-Silberberg and Schüpbach, 1993; Fig. 3). *fs(1)K10*, *squid*, *capu*, and *spire* are required for the proper localization of *grk* RNA, while the role of *cornichon* is still unknown (Neumann-Silberberg and Schüpbach, 1993). Thus, like Tor, DER activation is controlled by the localized and limited expression of a putative ligand. While these two points are clearly an important factor to providing patterning information via RTK's,

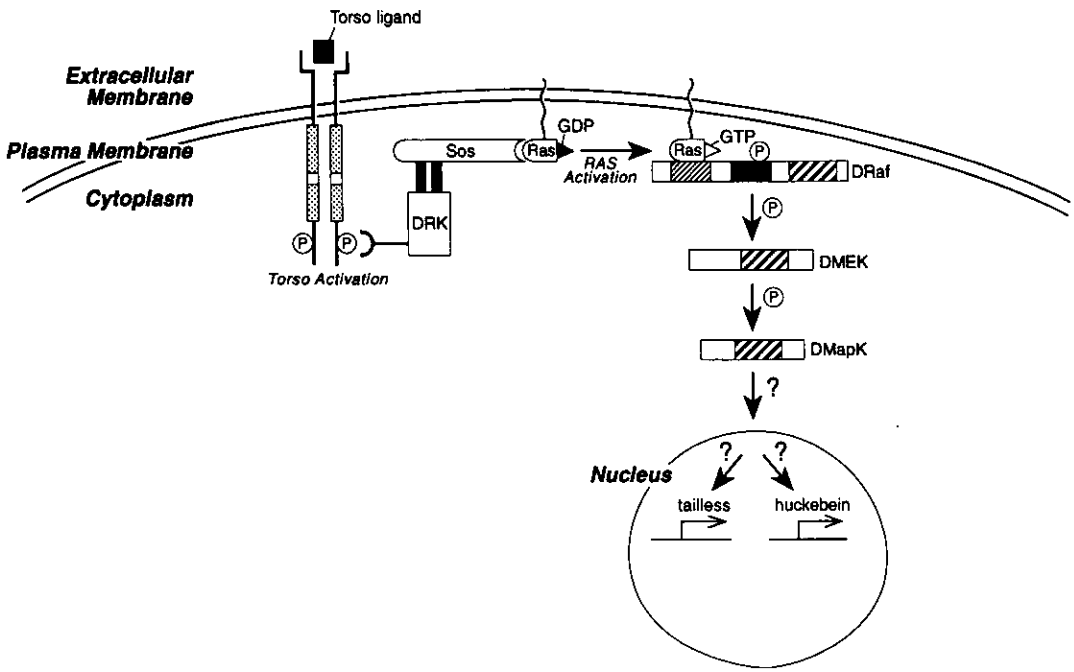

FIG. 6. Model of terminal class signaling. Ligand binding leads to the activation and intermolecular autophosphorylation of the Torso (Tor) receptor protein tyrosine kinase. These phosphotyrosine residues serve as docking sites for the SH2 domain of the adaptor protein downstream of receptor kinases (Drk). By virtue of its SH3 domains Drk links Sos to the activated receptor. This results in an increase in GTP-bound Ras and activation of Draf. Activation of Draf initiates a kinase cascade involving DMEK and DMAPK which leads to the transcriptional activation of the zygotic gap genes *tailless* and *huckebein*. It should be noted that the role of some of these components (Drk, and DMAPK) is still speculative. In addition, we have not depicted the Csw protein, although it is involved in terminal signaling. While Csw acts downstream of Tor, its positioning with respect to the other signaling components is still unclear.

the concentration, localization, and timing of receptor expression also appears to be a crucial element to ensuring proper patterning.

Experiments addressing these factors led to the simple concept of "ligand trapping" by RTK's. By restricting wild-type Tor expression to the central regions of the embryo Sprenger and Nüsslein-Volhard (1992), as well as Casanova and Struhl (1993), demonstrated that the Tor ligand is capable of diffusing in the perivitelline space from the terminal to the central regions of the embryo, where it then activates Tor (Sprenger and Nüsslein-Volhard, 1992; Casanova and Struhl, 1993). A similar situation also appears to exist for DER in d/v patterning. Whereas DER is normally activated in the dorsal-anterior region of the follicular epithelium, mislocalization of *grk* RNA within the oocyte results in activation of DER in ventral and posterior regions of the follicular epithelium (Neumann-Silberberg and Schüpbach, 1993; Duffy and Perrimon, in preparation). Thus, to maintain proper patterning Tor and DER must be present in the proper location, time, and in sufficient concentrations to ensure their activation is restricted to those regions dictated by the localized expression of their respective ligands. However, it should be noted that the rescue of telson structures (by *tor* RNA injection) is possible with 10-fold less RNA than that used to produce wild-type concentrations of Tor (Sprenger and Nüsslein-Volhard, 1992). In addition, increasing the dosage of Tor with P-element copies of a *tor* transgene has no apparent phenotypic effect (Casanova and Struhl, 1989). Thus, within these ranges, the concentration of Tor protein is not critical to patterning of the termini.

While Tor and DER RTK pathways are examples regarding the positive transduction of patterning information, scenarios in which RTKs (or other receptors) act as sinks by preventing a ligand from reaching its proper target may also exist. In fact, overexpression of kinase-inactive forms of Tor in wild-type embryos appears to act in such a fashion (Sprenger and Nüsslein-Volhard, 1992). These mutant Tor molecules inhibit terminal development by sequestering the limiting ligand and preventing activation of wild-type Tor molecules.

b. Determining Fates with RTKs: Gradients or Switches

The activation of Tor ultimately results in the determination of a field of cellular fates that give rise to the specialized structures located at the terminal regions of the larva. One critical question with respect to this and other RTK pathways regards the nature of the informa-

tion that is transduced from the membrane to the nucleus. Do signals transduced through RTKs serve solely to switch genes on or off, or are they capable of more precise (i.e., graded) effects on the levels of transcription? If graded effects are seen, how are they generated?

Tor activation has a number of effects at the transcriptional level: activation of *tll* and *hkb* expression and repression of *bcd* target genes, such as *hunchback (hb)* and *orthodenticle (otd).* Recently, Ronchi *et al.* (1993) have demonstrated that the repression of *hb* and *otd* at the anterior tip of the embryo involves the phosphorylation and inactivation of *bcd* in response to Tor activation. While the molecule directly responsible for phosphorylating *bcd* in response to Tor activity has not been identified, it is tempting to speculate that this role might be fulfilled by the *Drosophila* homologue of MAP kinase—*rolled.*

In contrast to the repression of *hb* and *otd* at the anterior tip, *tll* and *hkb* are activated at both termini in response to Tor activity. These two genes are expressed in overlapping, but not identical, domains with discrete boundaries. Within these domains each gene does not appear to be expressed in a gradient with a peak in expression at the most terminal regions; rather, their expression appears uniform within their respective domains. However, these observations alone do not provide significant insight as to whether Tor signaling acts as an on/off switch or is capable of providing different levels of activity.

Nevertheless, a variety of lines of evidence have been used to support the notion that the Tor pathway does produce a gradient of signaling information. Chief among these has been the ability to obtain strong, intermediate, and weak terminal class LOF and GOF phenotypes with different alleles or combinations of various terminal class members, including *tor*, *Draf*, and *csw* (Casanova and Struhl, 1989; Melnick *et al.*, 1993; Perkins and Perrimon, in preparation). With these phenotypic effects, concomitant shifts in the respective domains of expression of *tll* and *hkb* are also seen. First, such results indicate that different levels of Tor and terminal activity can be obtained. However, these results must be interpreted with caution as mutant proteins with reduced/impaired activity may represent unique situations which wild-type forms of these proteins may not be capable of achieving. Second, in various terminal GOF mutations, as well as with misdirected expression of *tor*, *tll* and *hkb* are expressed in the central region of the embryo. This indicates that the transcription factors responsible for activating *tll* and *hkb* expression must be ubiquitous and not in a graded fashion. All of these lines of evidence imply, but do not rigorously demonstrate that terminal activity produces a gradient of patterning activity.

Again, by drawing analogies to the DER and d/v patterning, insights into Tor signaling may be obtained. As with Tor, strong, intermediate, and weak ventralized phenotypes can be obtained with different alleles or combinations of various components of this pathway (Schüpbach, 1987; Clifford and Schüpbach, 1989; Forlani *et al.*, 1993). In addition, just like Tor, the activation of DER leads to the activation or repression of various loci (Brand and Perrimon, 1994; T. Schüpbach, personal communication; Duffy and Perrimon, in preparation). Yet unlike Tor, one of these target loci is expressed in a graded fashion in response to DER activity in the dorsal-anterior region of the follicular epithelium (Brand and Perrimon, 1994; T. Schüpbach, personal communication; Duffy and Perrimon in preparation). Is this gradient the result of different levels of DER activity, or is it the result of a more downstream mechanism?

As described in the previous section, whereas DER is normally activated in the dorsal-anterior region of the follicular epithelium; mislocalization of *grk* RNA results in activation of DER in ventral and posterior regions of the follicular epithelium (Neumann-Silberberg and Schüpbach, 1993; Duffy and Perrimon, in preparation). In response to this, no dorsal-anterior gradient of the marker is seen. Instead, expression is now evident in ventral and posterior follicular cells. In addition, activation of Draf in all follicle cells results in the uniform expression of this marker. These two results indicate that the transcription factor that activates this marker (in response to DER or Draf activity) is ubiquitiously distributed throughout the follicular epithelium and is not present in a gradient. Moreover, it strongly suggests that distinct cell fates can be achieved through DER (and other RTKs, like Tor) by regulating ligand concentration. The localized and limited release of a ligand (in this case Grk) coupled with saturating amounts of a RTK (in this case DER), "ligand trapping", combine to result in a gradient of RTK signaling activity as one moves away from the ligand source. The presence of unique levels of RTK activity in different cells then directs the specification of distinct cell fates. This indicates that RTK activity may be graded, but determination of cell fate is a threshold response to a discrete range of RTK activity.

Finally, the conservation of the RTK-Ras pathway among RTKs in many different eukaryotic systems, raises the question as to how the specificity of a particular RTK is produced. With the knowledge that different activities of this pathway are capable of directing unique outcomes it is tempting to speculate that distinct RTK-ligand interactions are capable of generating differing activities of the same downstream molecules. Carrying this notion one step further, perhaps one component in the evolution of the signaling specificity of

RTKs has revolved not around their ability to regulate distinct pathways, but rather their inherent ability to regulate the same pathway.

In spite of such speculative concepts, the analysis of the Tor RTK signaling pathway has nicely complemented and extended the biochemical, genetic, and molecular studies of other eukaryotic RTK pathways. The ability to isolate enhancers and suppressors of the currently identified components, as well as the possibility of testing specific molecules for *in vivo* effects on terminal development should continue to rapidly expand our knowledge of the role of RTKs in cellular communication and pattern formation. The use of approaches unique to a genetic system, like *Drosophila*, should continue to provide a necessary balance to the more biochemical approaches utilized in mammalian systems, again, with the hope that such studies will converge, revealing further insights into the mechanisms by which cells and, ultimately, organisms are patterned.

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