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The Torso Receptor Protein-Tyrosine Kinase Signaling Pathway: An Endless Story

Minireview

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One of the mechanisms by which cells respond to extracellular signals involves activation at the membrane of receptor protein-tyrosine kinases. Some of the molecules that transduce the signals generated by activated receptor protein-tyrosine kinases and that ultimately activate transcription factors have been extensively characterized, and a general picture has begun to emerge. Two general conclusions can be drawn from studies on the control of cell growth and differentiation of mammalian cells and genetic analyses of pathways that control cell fate determination in both Caenorhabditis elegans and Drosophila melanogaster. First, molecules involved in receptor protein-tyrosine kinase signaling have been highly conserved during evolution. Second, and perhaps more surprisingly, it appears that all receptor protein-tyrosine kinases activate a common set of molecules that includes $p21^{ras}$, Ras-associated regulatory proteins, Raf, MAP kinase (MAPK), and Mek (MAPK or Erk kinase). Here, our current understanding of the Drosophila torso (tor) receptor protein-tyrosine kinase signaling pathway is described. The torso pathway, together with the Drosophila sevenless pathway required for photoreceptor R7 development and the C. elegans Let-23 pathway required for vulval development, has been used genetically to dissect a receptor protein-tyrosine kinase signaling pathway.

Biological Role of the Torso Signaling Pathway

Genetic and embryologic analyses of the early Drosophila embryo have identified the terminal system, which is involved in cellular determination of both the tail and unsegmented head regions (Nüsslein-Volhard et al., 1987). This system differs from the anterior (*bicoid*) and posterior (*nanos*) body patterning systems, which operate along the anteroposterior axis to determine segmented head, thoracic, and abdominal cell fates: the terminal system controls patterning in two noncontiguous embryonic domains. While both the anterior and posterior systems use RNA localization strategies to generate protein gradients that control the expression of transcription factors, the terminal system uses a receptor protein-tyrosine kinase signal transduction pathway (St Johnston and Nüsslein-Volhard, 1992; Figure 1). A feature common to these body patterning systems is that they operate during the syncytial blastoderm stage of embryogenesis, i.e., prior to cellularization. At this time, molecules can freely diffuse, and a single morphogen-generated signal is able to instruct groups of rapidly dividing nuclei such that large domains of the body plan can be defined.

The receptor protein-tyrosine kinase that triggers terminal cell fate development is encoded by the *torso* (*tor*) gene. The structure of the torso protein is reminiscent of the mammalian platelet-derived growth factor (PDGF) receptor protein-tyrosine kinase (Sprenger et al., 1989). Torso is necessary and sufficient for the determination of terminal cell fates. This has been shown by analyses with gain-of-function *torso* mutations (tor^{gof}) as well as injection of mRNA encoding an activated mutant torso protein into syncytial embryos (Sprenger and Nüsslein-Volhard, 1992).

Like the anterior and posterior systems, the signal generated by torso ultimately controls the activation of specific transcription factors. The best-characterized downstream component of the torso signaling pathway is the product of the *tailless* (*tll*) gene, which encodes a putative transcrip-

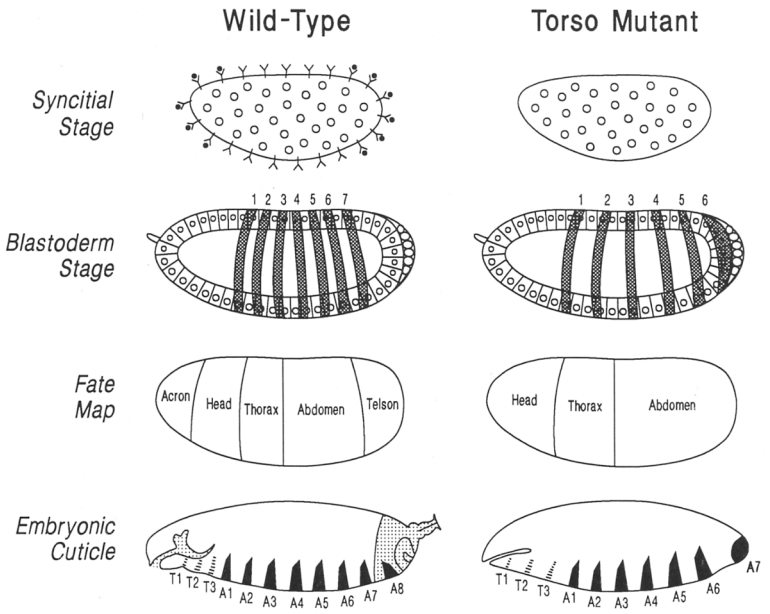

Figure 1. Torso Signaling Pathway during Embryonic Development

In the wild-type syncytial embryo, the ubiquitous torso receptor protein-tyrosine kinase, which is locally activated at each terminus by an activity present in the perivitelline fluid, triggers a signal transduction pathway that controls gene expression at the termini. Torso signaling, together with the other body-forming systems, defines the regions from which the larval body regions will arise (i.e., a fate map). At the cellular blastoderm stage, the overall body plan (shown by the expression of the pair rule gene *fushi tarazu* in seven stripes) is already determined. In a *torso* mutant embryo, the terminal cuticular regions, which include part of the head skeleton and all structures posterior to abdominal segment 7 (shaded region in the wild-type embryonic cuticle) (Nüsslein-Volhard et al., 1987), are deleted as a result of the reorganization of the body plan (indicated here by the presence of only six *fushi tarazu* stripes).

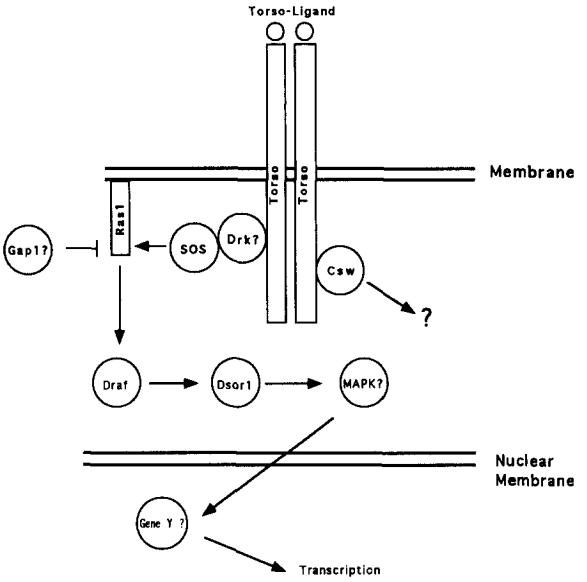

Figure 2. Cascade of Events Triggered by Torso Activation

Activation (→) and negative (—|) regulatory effects are shown.

tion factor of the steroid hormone superfamily (Pignoni et al., 1990). Mutations in *tailless* result in a cuticular phenotype reminiscent of null *torso* mutations and in genetic epistasis experiments can suppress the tor^{gof} cuticular phenotype (Klingler et al., 1988). In addition, *tailless*, which is expressed in wild type at each embryonic terminus, is ubiquitously expressed in tor^{gof} mutations (Steingrímsson et al., 1991). In the tail region, *tailless*, in concert with at least one other putative transcription factor, *huckebein* (*hkb*), controls the spatial expression of additional transcription factors, such as *forkhead*, *hunchback*, and *fushi tarazu*.

Patterning of the unsegmented head region is more complex, since inputs from both the *torso* and the *bicoid* systems are required to control the spatial expression of downstream target genes, such as *orthodenticle* and *hunchback* (Finkelstein and Perrimon, 1990; Pignoni et al., 1992; Ronchi et al., 1993). These downstream transcription factors that are terminally expressed ultimately initiate developmental programs that result in the differentiation of both head and tail structures (Perkins and Perrimon, 1991).

Torso Activation: A Common Developmental Strategy?

Recent studies have shed light on the mechanisms that control torso activation (Sprenger and Nüsslein-Volhard, 1992; Casanova and Struhl, 1993). Activation of the receptor is controlled by a terminal activity, i.e., a ligand, present in the fluid-filled perivitelline space that surrounds the embryo. The torso protein, which is uniformly distributed along the egg cell membrane (Casanova and Struhl, 1989), becomes activated at each terminus, where the terminal activity is localized. In the absence of torso receptor, this activity is able to diffuse freely within the perivitelline space. The molecular mechanism underlying torso activation is still unclear. However, analyses of mutations with phenotypes similar to *torso* have identified four genes that are required for the generation of this terminalizing activity (St Johnston and Nüsslein-Volhard, 1992). One of them, *torso-like* (*tsl*), is required in two distinct subpopulations of follicle cells located at each terminus of the developing oocyte (Stevens et al., 1990). Therefore, *torso-like* may encode the torso ligand or an activity that is required to specify the regions in which the torso receptor is activated.

The observation that the torso ligand, in the absence of the receptor, can freely diffuse in the perivitelline space (Sprenger and Nüsslein-Volhard, 1992; Casanova and Struhl, 1993) indicates that the receptor not only transduces the spatial signal but is also required to restrict diffusion of its ligand. This dual receptor function may be a rather common strategy. For example, in the similar process of controlling dorsal–ventral axis determination, the Toll receptor, which is uniformly expressed at the egg cell membrane, becomes activated in response to a ventralizing activity/ligand. In the absence of Toll, the ventralizing activity/ligand can diffuse freely within the perivitelline space (Stein et al., 1991).

Torso Transducers Are Shared with Other Receptor Protein-Tyrosine Kinases

Once bound to its ligand, the torso receptor protein-tyrosine kinase most likely dimerizes and either auto- or transphosphorylates intracellular torso tyrosine residues (Cantley et al., 1991). The activated torso receptor protein-tyrosine kinase then initiates a cascade or pathway of events (Figure 2). The first characterized component of this pathway is *l(1)pole hole* (Perrimon et al., 1985), which encodes the Drosophila homolog of the mammalian Raf-1 serine/threonine kinase (and hence is also named D-raf) (Ambrosio et al., 1989; Nishida et al., 1988). *D-raf* activity is required downstream of torso, since it is necessary for the tor^{gof} phenotype (Ambrosio et al., 1989). It is required upstream of tailless, since *tailless* is not expressed in eggs lacking *D-raf* activity (Pignoni et al., 1992).

Activation of D-raf by the torso signal involves the guanine nucleotide–binding protein $p21^{ras}$ encoded by *Ras1*. Injection of both activated and dominant negative forms of $p21^{ras}$ protein into precellular (syncytial stage) embryos of various genotypes has demonstrated that Ras1 relays the torso signal to D-raf (Lu et al., 1993). In addition, germline mosaic analyses of mutations in the gene *Son of sevenless* (*Sos*), which encodes a Drosophila guanine nucleotide releasing (or exchange) factor (GRF) has demonstrated that Sos acts to positively regulate Ras1 activity in torso signaling. In support of these results, mutations that suppress a tor^{gof} mutation have been isolated in both the Drosophila *Ras1* and *Sos* genes (Doyle and Bishop, 1993).

A search for second-site suppressors of a weak *D-raf* allele has led to the identification of a putative target of D-raf. A gain-of-function mutation, Dsor1, bypasses the requirement for torso and D-raf activity (Tsuda et al., 1993). Molecular characterization of a revertant of Dsor1 identified the Drosophila homolog of the tyrosine/threonine kinase Mek, strongly suggesting that Dsor1 is an activating mutation of Mek. The current model postulates that Dsor1

controls, possibly through the serine/threonine kinase MAP kinase, the specific activation of a transcription factor (*gene Y*) at each egg terminus, which in turn controls the spatial expression of the terminal genes *tailless* and *huckebein*. Possibly, MAP kinase links the signal transduction pathway to the nuclear transcription factors, since it has been shown to translocate to the nucleus and phosphorylate transcription factors such as Jun and Elk-1 (Pulverer et al., 1991; Chen et al., 1992; Marais et al., 1993).

In addition, the *corkscrew* (*csw*) gene, which encodes a putative non-receptor protein-tyrosine phosphatase as well as two Src homology 2 (SH2) domains, has been shown to operate positively in torso signaling (Perkins et al., 1992). A mammalian protein-tyrosine phosphatase, similar in structure and sequence to corkscrew (named SH-PTP2 [Freeman et al., 1992], PTP1D [Vogel et al., 1993], or Syp [Feng et al., 1993]) has been shown to bind to activated receptor protein-tyrosine kinases and become phosphorylated on tyrosine residues. By analogy, the putative corkscrew protein-tyrosine phosphatase may bind to the activated torso receptor protein-tyrosine kinase, after which corkscrew activity becomes modified in response to a change in its state of tyrosine phosphorylation. Other potential targets or substrates of corkscrew have yet to be identified. Injection of activated $p21^{ras}$ into precellular *corkscrew* embryos rescues their terminal defects (Lu et al., 1993), suggesting that corkscrew operates upstream of Ras1. However, these results have to be viewed cautiously, since they do not rule out the presence of two partially redundant pathways that converge onto a more downstream component of the torso pathway. Clearly, further biochemical characterization is needed to clarify the function of corkscrew in torso receptor protein-tyrosine kinase signaling.

Taken together the results described above indicate that Sos, Ras1, D-raf, Dsor1, and corkscrew positively transduce the spatial signal in response to the activated torso receptor protein-tyrosine kinase. Comparisons of the molecules involved in the torso signaling pathway with molecules in other receptor protein-tyrosine kinase pathways (Table 1) suggest that all receptor protein-tyrosine kinases activate a common set of proteins. To date, no molecules have been identified that are specific to a single receptor protein-tyrosine kinase pathway.

Prospects

Comparisons of the molecules involved in receptor protein-tyrosine kinase signaling lead to the preliminary conclusion that the qualitative output of all receptor protein-tyrosine kinases is similar. It is possible that most receptor protein-tyrosine kinases simply act as on/off switches and that the specific responses elicited by each simply reflect the variety of transcriptional regulators present in the various cell types. However, this idea does not hold true for PC12 cells: stimulation of the Trk receptor protein-tyrosine kinase by nerve growth factor elicits differentiation, but stimulation of the ErbB receptor protein-tyrosine kinase in the same cells by epidermal growth factor elicits a different response: cell proliferation (Tishler and Greene, 1975; Huff et al., 1981). This suggests that a single cell is able to distinguish the signals generated by various activated receptor protein-tyrosine kinases (Chao, 1992). Whether the specificity in the case of the PC12 responses resides in qualitative or quantitative differences remains unclear.

Since many components are shared between receptor protein-tyrosine kinase signaling pathways, it is important to determine the extent of this conservation. For example, an adaptor protein identified as Sem-5, Drk, or Grb2 operates in the Let-23, sevenless, and mammalian signaling pathways, respectively, but has yet to be shown to play a role in the torso pathway (Table 1). In addition, a number of proteins, such as phospholipase C-γ, phosphatidylinositol 3-kinase, and $pp60^{c\text{-}src}$, that have been implicated in mammalian receptor protein-tyrosine kinase pathways (Cantley et al., 1991) have, rather surprisingly, not yet turned up in invertebrate studies. Systematic analyses of the function of each of these proteins in multiple pathways may ultimately reveal that some components are indeed specific to one or only a few of the receptor protein-tyrosine kinase signaling pathways.

One property unique to the torso signaling pathway is the ability to test directly (by injections of either RNA or protein into precellular, syncytial stage embryos) the effects of specific molecules on terminal development. The need for mutations in the genes in the signaling pathway is bypassed by the ability to inject dominant negative and/or activated forms of specific signaling proteins as well as specific compounds that interfere with the functions of the signaling proteins. Injection of interfering or activating molecules in the early embryo also provides a tractable assay to test various drugs that might alter the efficiency of the signaling pathway. The output of torso signaling in such experiments can easily be monitored by following the do-

Table 1. Molecules Involved in Receptor Protein-Tyrosine Kinase Signaling

Activity	Torso Pathway	Sevenless Pathway	Let-23 Pathway	PDGF Receptor Pathway
Receptor protein-tyrosine kinase	Torso	Sevenless	Let-23	PDGF receptor
Adaptor	ND	Drk	Sem-5	Grb2
Protein-tyrosine phosphatase	Corkscrew	ND	ND	SH-PTP2
Guanine nucleotide releasing factor	Sos	Sos	ND	Sos1, Sos2
GTPase-activating protein	ND	Gap1	ND	RasGAP
Guanine nucleotide–binding protein	Ras1	Ras1	Let-60	$p21^{ras}$
Ser/Thr kinase	D-raf	D-raf	Lin-45	Raf1
Thr/Tyr kinase	Dsor1	ND	ND	Mek
Ser/Thr kinase	ND	ND	ND	MAP kinase

ND, not yet determined. For the role of each component in the sevenless signaling pathway, see Dickson et al. (1992); Olivier et al. (1993); Simon et al. (1991, 1993). For Let-23 signaling, Pawson (1992); Han et al. (1993). For PDGF receptor and other mammalian receptor protein-tyrosine kinase signaling pathways, Cantley et al. (1991); Roberts (1992); McCormick (1993).

mains of expression of target transcription factors such as tailless or huckebein.

Since the downstream signaling molecules characterized to date are used in more than one receptor protein-tyrosine kinase signaling pathway, mutations in the genes that encode them will most likely result in lethality. Indeed, the discovery of the role of genes such as *D-raf* and *corkscrew* in torso signaling was dependent on screens that rely on the examination of the maternal effects of zygotic lethal mutations (Perrimon et al., 1989). These screens have been performed for the X chromosome; screening the rest of the genome will most likely lead to the identification of additional members of the torso signaling pathway. A powerful alternative approach to isolate components of a signal transduction pathway is to conduct screens in a sensitized genetic background (Simon et al., 1991). For example, in a screen for dominant suppressors of a gain-of-function *torso* mutation, at least seven complementation groups have been identified, among which are *Sos* and *Ras1* (Doyle and Bishop, 1993).

In conclusion, studies on the torso receptor protein-tyrosine kinase signaling pathway (as well as the sevenless and Let-23 pathways) have revealed that the molecules involved in transducing the signal are similar to those identified from studies in mammalian cells. The genetic approach to receptor protein-tyrosine kinase signaling has contributed to an understanding of the functional interactions between the signaling molecules. When combined with their known biochemical properties, this provides an ever clearer picture of the intricacies of these pathways. Further genetic and biochemical dissection of the components involved in torso signaling combined with comparisons of this pathway with others are providing exciting lines of research.

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