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One of the amazing features of *Drosophila* development is the breathtaking speed of its embryogenesis. The fertilized egg develops into a hatching larva with a complex form and structure in a period of about 24 hours. The remarkable speed of this process is made possible because the foundation for embryonic pattern formation (dorsal-ventral and anterior-posterior) is established during oogenesis (*1*). For developmental biologists studying the earliest events of embryonic pattern formation, the task is to understand how the information deposited in the egg by the mother is translated into the polarized structures of the emergent first-instar larva. Recent work from the Casanova lab (*2*) may provide one of the missing links in our understanding of how the structures of the anterior-posterior axis of the *Drosophila* embryo are defined. Their data provide evidence that the maternal-effect gene *trunk* encodes a putative growth factor ligand, and that extracellular processing of this ligand at the anterior and posterior termini of the developing embryo is likely to set in the motion the signaling pathway that defines the two ends.

The *trunk* gene is a member of a group of maternal-effect genes that are required for the development of the most anterior and posterior structures (the acron and telson, respectively) of the embryo (*3*). Collectively, this group of genes is known as the terminal class. Mothers carrying a mutation of one of the terminal-class genes produce embryos that lack the most anterior structures of the head, as well as the structures normally positioned posterior to the seventh abdominal segment. In addition to this loss-of-function phenotype, mutation of one of the other terminal-class genes, *torso*, can also produce gain-of-function alleles (*4*). Embryos derived from females carrying these mutations exhibit a phenotype opposite to the one described above—that is, an expansion of the terminal regions at the expense of the segmented middle portion of the body.

The identification of Torso as a member of the receptor protein tyrosine kinase (RTK) class of proteins (*5*) and the demonstration that it was uniformly distributed throughout the embryonic membrane (*6*) provided a model for understanding both the loss-of-function terminal class and *torso* gain-of-function phenotypes. This model predicts that Torso is activated by a ligand that is restricted to the anterior and posterior ends of the embryo during normal development. The *torso* gain-of-function alleles were presumed to encode ligand-independent forms of the molecule, a presumption supported by the demonstration that the mutant lesions localized to the extracellular, putative ligand-binding portion of the molecule (*7*). Torso activation sets in motion a typical Ras-Raf-MAPK (mitogen-associated protein kinase) cascade (*8*) that ultimately determines the fate of the cells developing in the parts of the embryo where receptor activation has occurred.

Despite a wealth of information regarding the downstream effectors of the pathway, the identity of the Torso ligand and the mechanism underlying its localization have been elusive. An early clue in solving this mystery was the demonstration that expression of *torso-like*, a terminal-class gene that acts upstream of *torso*, is required in the somatic follicle cells that surround the developing oocyte (*9*). Furthermore, mosaic analysis showed that *torso-like* expression is specifically required in follicle cells that lie at the poles of the developing oocyte, suggesting that localized expression of *torso-like* in the follicular epithelium is responsible for the polar restriction of Torso activity. Consistent with this model, molecular cloning of *torso-like* showed that it is specifically expressed in two polar subpopulations of follicle cells (*10*, *11*) (Fig. 1), and that ectopic expression of *torso-like* throughout the follicle cell layer leads to a phenotype reminiscent of that produced by *torso* gain-of-function alleles (*10*). Because *torso-like* appears to encode a secreted molecule, albeit of unknown function, it was hypothesized that Torso-like may correspond to the ligand for Torso. However, the subsequent cloning of *trunk* (*12*), another terminal-class gene acting upstream of Torso, identified a different contender for the title of Torso ligand. *trunk* also encodes a putative secreted molecule, but in contrast to *torso-like*, it is expressed in the germ line (nurse cells and oocyte), rather than in the somatic component of the ovary.

A recent publication by Casali and Casanova investigates two putative protease cleavage sites in Trunk (*2*). The use of the most NH_2-terminal site would generate a COOH-terminal cleavage product of 159 amino acids, whereas use of the other site would generate a COOH-terminal product of 108 amino acids. To test the ability of these Trunk cleavage products to activate Torso, the authors used recombinant techniques to engineer constructs that would contain a signal peptide-coding region fused directly to the predicted Trunk cleavage products. Injection of RNA encoding either of these constructs, $Trunk^{C159}$ or $Trunk^{C108}$, into the progeny of *trunk* mutant females partially rescued the terminal-class phenotype in a small number of embryos. The $Trunk^{C108}$ construct also induced the development of terminal structures in embryos derived from females mutant for the terminal-class genes *torso-like*, *fs(1)Nasrat*, and *fs(1)polehole*, but did not rescue the progeny of females mutant for *torso*. Thus, removal of the NH_2-terminus of Trunk eliminated the requirement for the upstream terminal-class genes; however, the rescue remained *torso* dependent, an important prerequisite for a potential Torso ligand. Results similar to the ones described above were also obtained when $Trunk^{C108}$ expression was accomplished transgenically, with an expression system that allows maternal germ line expression (*13*). In this way, it was possible to generate embryos expressing larger and more uniformly distributed amounts of $Trunk^{C108}$ than would be pos-

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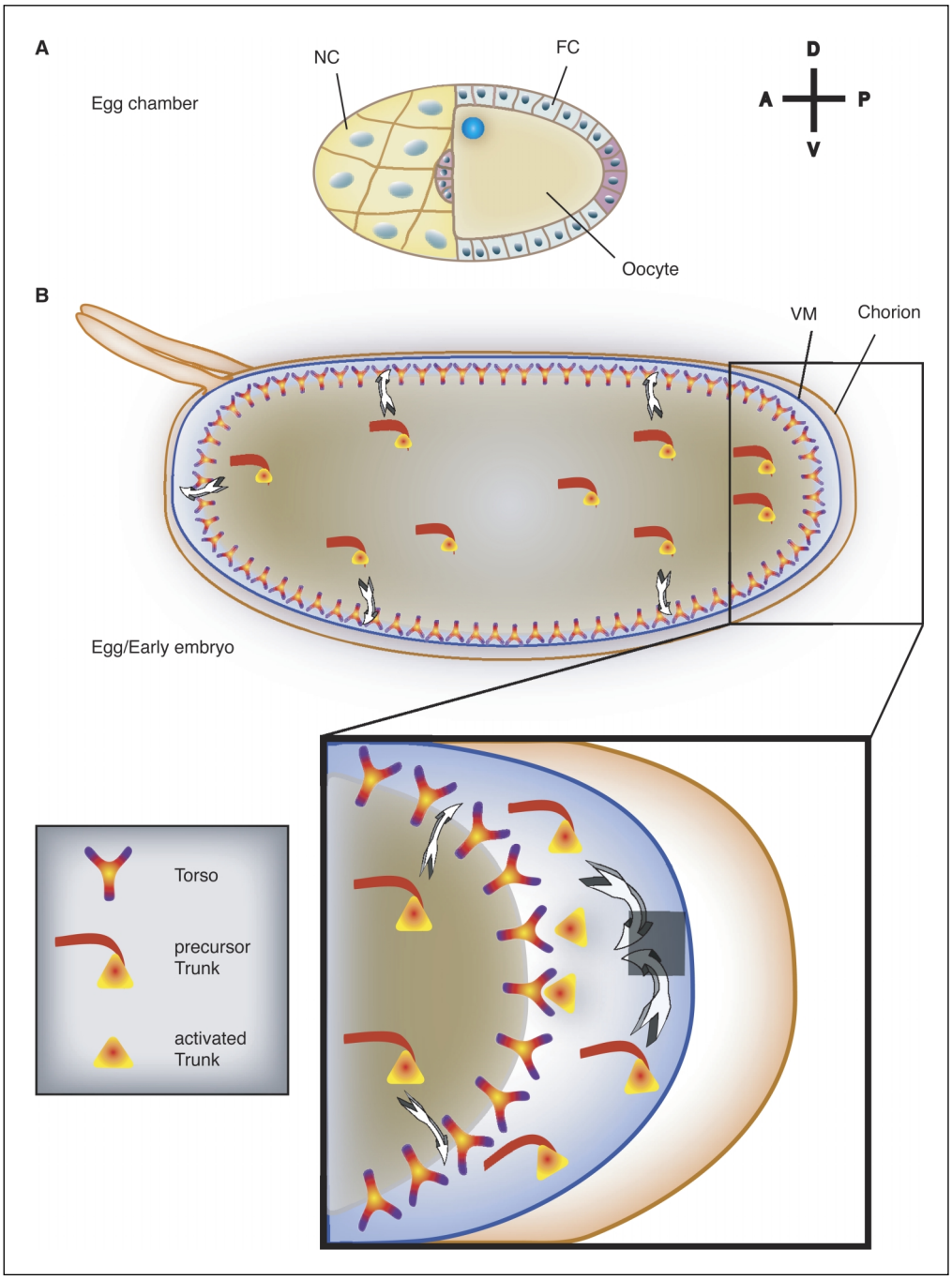

Fig. 1. Events during oogenesis and embryogenesis that influence patterning of the embryonic termini. (**A**) In the stage 10 egg chamber, a complex of 15 nurse cells (NC) is situated at the anterior end of the single large oocyte, which is also enveloped in an epithelium of somatically derived follicle cells (FC). Specialized subpopulations of follicle cells called polar cells (shaded in pink), located at the anterior and posterior end of the oocyte, express the terminal-class gene *torso-like* and provide a localized cue for terminal patterning during embryogenesis. D, dorsal; P, posterior; V, ventral; A, anterior. (**B**) In the developing embryo, the Torso receptor is uniformly distributed throughout the embryonic membrane. The Trunk protein is produced in a precursor form that is secreted uniformly into the perivitelline space between the embryonic membrane and the inner layer of the egg shell, called the vitelline membrane (VM). At the anterior and posterior ends of the embryo, Trunk is converted into an active ligand for Torso, to which it binds, thereby activating the signal transduction pathway that defines the termini of the embryo (see boxed inset). The mechanism underlying localized processing of Trunk remains unknown. However, the Torso-like protein is likely to be a component of this black box.

sible with RNA injection. These embryos exhibited a phenotype similar to that of the *torso* gain-of-function phenotype: deletion of middle-body segments and a concomitant expansion of the termini. This suggests that the precleaved form of the Trunk protein can be active in nonpolar regions of the embryo and that the formation of the Trunk cleavage product must be restricted to the poles for development to proceed normally.

The ability of Trunk to activate Torso in the absence of the products of other terminal-class genes strongly suggests that *trunk* encodes the ligand for Torso and implies that the function of the other upstream genes may be to bring about the pole-restricted cleavage of Trunk. As noted by Casali and Casanova, the existence of a *trunk* mutation that affects one of the putative cleavage sites suggests that cleavage is physiologically relevant and that the ability of the cleaved variants to activate Torso is not an artifact. It is not clear why there are two protease cleavage sites in Trunk; the authors suggest that localized cleavage of Trunk may be a multistep process, with cleavage at position 67 a prerequisite for cleavage at position 119.

Although the results from Cassali and Casanova provide a convincing argument for the identification of Trunk as the ligand for Torso, some important details remain to be elaborated.

For one, Trunk has not been shown to bind directly to Torso, and although Trunk constructs lacking the NH_2-terminus are sufficient to activate Torso, a cleaved form of Trunk has not been identified in vivo, nor has a terminal-class gene encoding a putative protease been identified. It is possible that Trunk activation is accomplished in another way. For example, Trunk may normally exist in an inactive conformation, conferred by the presence of the regulatory NH_2-terminal domain. A physical interaction with one or more of the other terminal-class gene products (for example, Torso-like) might alter the conformation of Trunk and facilitate its interaction with Torso. Although the relatively inefficient reported rescue of terminal patterning by the precleaved forms of Trunk may have resulted from poor stability of the injected RNA or from other technical issues, it is tempting to speculate that activation of Torso may involve more than simple processing of Trunk. However, if Trunk processing can ultimately be demonstrated in vivo, the recent sequencing of the *Drosophila* genome has provided a list of 450 potential genes encoding peptidases that might do the job (*14*).

If Trunk is the ligand, what role does Torso-like play in this pathway? So far, no definitive explanation for its function has emerged, although it seems certain that Torso-like provides the spatial specificity for Torso activation. Torso-like protein deposited into the embryonic membrane (or the eggshell) could potentially mediate the presentation of Trunk to a proteolytic activity. Although Torso-like does not exhibit any strong similarities to known gene products, it has sequence similarity to the membrane attack complex/perforin domain that is present in many membrane pore-forming proteins (*15*). Unfortunately, the significance of this similarity, if any, is unclear.

During the establishment of dorsal-ventral polarity, the spatially localized event that defines the ventral proteolytic activation of Spätzle, the ligand for the transmembrane receptor Toll, is the expression of the Pipe protein in a ventral subpopulation of follicle cells (*16*). The similarity of Pipe to vertebrate glycosaminoglycan sulfotransferases suggests that an oligosaccharide modified by Pipe and deposited ventrally in the egg provides the cue for serine protease activation. Whether a similar process regulates Trunk processing, or whether the direct action of Torso-like in the egg obviates such a function, remains an interesting question for investigation.

Finally, the molecular identification of all members of the terminal class of genes is not yet complete. Two of them, *fs(1)Nasrat* and *fs(1)Polehole*, remain to be cloned. It is to be hoped that characterization of these genes will further elucidate how the localized activation of Torso by Trunk is accomplished.

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