Current Biology, Vol. 13, 1058–1063, June 17, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/S0960-9822(03)00379-8

The *Drosophila* Embryonic Patterning Determinant Torsolike Is a Component of the Eggshell

Leslie M. Stevens,[1,3,*] Dirk Beuchle,[1,4] Jennifer Jurcsak,[1] Xianglan Tong,[1] and David Stein[2,3]

[1]Department of Developmental and Molecular Biology
[2]Department of Molecular Genetics
Albert Einstein College of Medicine
1300 Morris Park Avenue
Bronx, New York 10461

Summary

The development of the head and tail regions of the *Drosophila* embryo is dependent upon the localized polar activation of Torso (Tor), a receptor tyrosine kinase that is uniformly distributed in the membrane of the developing embryo [1, 2]. Trunk (Trk), the proposed ligand for Tor, is secreted as an inactive precursor into the perivitelline fluid that lies between the embryonic membrane and the vitelline membrane (VM), the inner layer of the eggshell [3, 4]. The spatial regulation of Trk processing is thought to be mediated by the secreted product of the *torsolike (tsl)* gene, which is expressed during oogenesis by a specialized population of follicle cells present at the two ends of the oocyte [5, 6]. We show here that Tsl protein is specifically localized to the polar regions of the VM in laid eggs. We further demonstrate that although Tsl can associate with nonpolar regions of the VM, the activity of polar-localized Tsl is enhanced, suggesting the existence of another spatially restricted factor acting in this pathway. The incorporation of Tsl into the VM provides a mechanism for the transfer of spatial information from the follicle cells to the developing embryo. To our knowledge, Tsl represents the first example of an embryonic patterning determinant that is a component of the eggshell.

Results and Discussion

Although it has been reported that Tsl protein is present on the embryonic membrane [6], we were not able to replicate this result. Instead, using affinity-purified anti-Tsl antibodies, we detected strong staining of Tsl protein at the anterior and posterior poles of the VM, the inner layer of the eggshell, of 0- to 2-hr-old embryos (Figures 1A and 1B). These stainings were carried out by dechorionating embryos and attaching them, still contained within their VMs, onto glass microscope slides. We then used a needle to penetrate the VMs and release the embryos; the empty VMs were left attached to the slide and were fixed and then incubated with anti-Tsl antibodies (see the Experimental Procedures). Tsl staining could only be detected if the antibodies had access to the inner face of the VM; there was no staining of intact VMs (with the embryo still inside). This indicates that Tsl protein is present on the inside of the VM and is thus capable of interacting with components of the perivitelline fluid. No signal was present on the VMs of embryos derived from females homozygous for $tsl^{PZRev32}$, a protein null allele of *tsl* (see the Experimental Procedures); this finding indicates that the staining is specific for Tsl protein (Figures 1D and 1E). The binding of Tsl to the VM is quite stable, as the polar staining pattern of VMs from 7- to 9-hr-old embryos (Figure 1C) was equivalent in intensity to that of 0- to 2-hr-old embryos stained in parallel (Figure 1A).

The distribution of Tsl on the VM of embryos maternally mutant for *tor* or *trk* was identical to that observed for wild-type embryos (Figures 2A and 2B), as would be expected given their proposed downstream functions as receptor and ligand, respectively. In addition to *trk* and *tsl*, two other maternally expressed genes, *female sterile(1)Nasrat [fs(1)N]* and *female sterile(1)pole hole [fs(1)ph]* [7–9], are required for the activation of the Tor receptor. Although females homozygous for the hypomorphic alleles $fs(1)ph^{1901}$ and $fs(1)N^{211}$ produce embryos with the terminal class phenotype of head and tail defects, females mutant for stronger alleles of these two genes produce collapsed eggs, suggesting an additional role in VM formation. Consistent with this finding, it has recently been reported that stronger alleles of *fs(1)ph* and *fs(1)N* disrupt cross-linking of the VM [10, 11], an important step in eggshell formation. Both *fs(1)ph* and *fs(1)N* encode large extracellular proteins that are expressed in the germline during oogenesis and coat the oocyte surface [11]. The activities of *fs(1)N* and *fs(1)ph* have been reported to be required for the accumulation and stabilization at the oocyte surface of an epitope-tagged version of the Tsl protein expressed in its normal domain at the poles of the follicle [11]. When we used anti-Tsl antibodies to stain the VMs of embryos from mothers mutant for $fs(1)ph^{1901}$ and $fs(1)N^{211}$, Tsl was still detected in a polar cap, but the staining was less intense and appeared to be distributed over a larger region than in wild-type VMs (Figures 2C and 2D). Thus, in eggs from $fs(1)ph^{1901}$ and $fs(1)N^{211}$ mutant females, Tsl protein is retained on the VM, but it appears to spread from the poles and is not as highly concentrated at the ends as in wild-type eggs.

Furriols et al. [12] have shown that *tsl* expressed in the germline can rescue the loss-of-function phenotype of embryos derived from *tsl* mutant mothers and, at higher levels, produces the segmentation defects characteristic of the gain-of-function phenotype caused by ectopic Tor activation. However, these effects are not seen in the embryos of *fs(1)ph* and *fs(1)N* mutant mothers expressing *tsl* in the germline [12]. Although the concentration of polar Tsl protein is reduced on the VMs

*Correspondence: flylady@mail.utexas.edu
[3]Present address: Department of Molecular Cell and Developmental Biology and Institute for Cellular and Molecular Biology, 2401 West 24th and Speedway, University of Texas at Austin, Austin, Texas 78712.
[4]Present address: Max-Plank-Institut für Entwicklungsbiologie, Spemannstrasse 35/III, 72076 Tübingen, Germany.

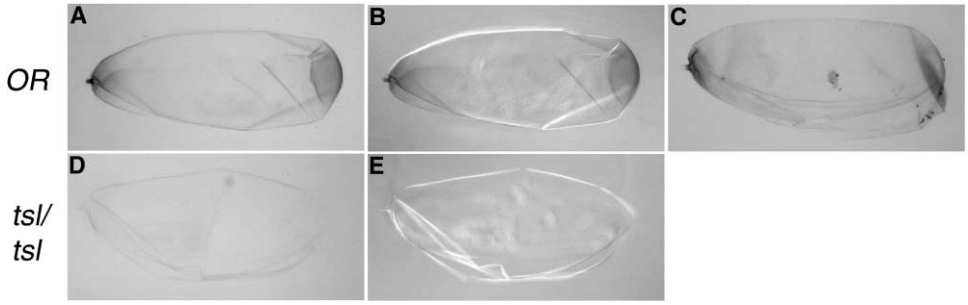

Figure 1. Tsl Protein Is Localized to the Poles of the VM

(A–E) (A, C and D) Bright-field and (B and E) DIC images of VMs stained with antibodies against Tsl. In this and all subsequent figures, images are oriented with anterior structures to the left. Maternal genotypes are indicated. VMs of 0- to 2-hr eggs derived from (A and B) wild-type mothers exhibit staining at the poles, while the VMs of eggs derived from (D and E) mothers homozygous mutant for *tsl*^{PZRev32} lack staining. (C) In wild-type embryos, high levels of localized Tsl are still detected at least 9 hr after egg deposition.

of *fs(1)ph* maternal mutants (Figures 2C and 3B), we were able to achieve uniformly high levels of Tsl protein on the VMs of these embryos by using the Gal4/UAS system [13] to ectopically express *tsl* in the follicle cell layer of mutant mothers (Figure 3F). In the progeny of otherwise wild-type females, high levels of Tsl protein on the VM (Figure 3D) produced a strong gain-of-function phenotype in the embryo, in which the head and tail structures were present but the segmented thoracic and abdominal regions of the embryo were disrupted (Figure 3C). In contrast, despite high levels of Tsl on their VMs, the embryos from *fs(1)ph/fs(1)ph* homozygous mutant mothers ectopically expressing *tsl* exhibited a terminal loss-of-function phenotype (Figure 3E) indistinguishable from that of embryos from *fs(1)ph* mutant mothers not misexpressing *tsl* (Figure 3A). In these embryos, the segmented region of the embryo is normal, but the head is disrupted and structures posterior to abdominal segment 8 are deleted. Similar results were obtained for *fs(1)N* maternal mutants (data not shown). These results indicate that the loss-of-function phenotype seen in embryos from *fs(1)ph*^{1901} and *fs(1)N*^{211} mothers is not due simply to decreased amounts of Tsl protein on the VM, but rather reflects a specific requirement for Polehole and Nasrat activities in order for Tsl to exert its function.

The ability of ectopically expressed Tsl to produce an embryonic phenotype similar to that of constitutively active Tor has been interpreted to mean that Tsl is capable of functioning ectopically, and that consequently, the restriction of *tsl* expression to the poles of the follicle is critical for the production of a localized Tor ligand [5, 6]. However, because the ligand for Tor is diffusible, the spatial parameters of Tor activation are determined by the concentration and distribution of Tor protein in the embryonic membrane relative to the amount of ligand processed at the poles [14, 15]. In the work described below, we present evidence that even when *tsl* is expressed ectopically, it is active only in the polar regions. Thus, the *tsl* gain-of-function phenotype is likely the result of diffusion of excess ligand from the poles. In these experiments, we expressed *tsl* at low levels in the female germline; these low levels resulted in the uniform distribution of Tsl protein in the VM of the embryonic progeny (Figure 4H). *tsl* mutant females carrying this construct, termed *CBBtsl*, produced some embryos in which the terminal structures were completely restored (Figure 4G). Despite the complete rescue of terminal cuticular structures, many of these embryos did not exhibit the segmentation defects associated with uniform Tor activation [16, 17]. These segmentation defects are caused by ectopic expression of *tailless* (*tll*), the terminal region gap gene [18], which at high levels can interfere with the expression of central gap genes such as *Krüppel* (*Kr*) [19]. Consistent with the relatively normal cuticular phenotypes of the embryonic progeny of *tsl* mutant mothers carrying *CBBtsl*, *tll* expression in these embryos was found to be restricted to the ends of the embryo (Figure 4I); this finding suggests that Tor receptor activation was restricted to the polar regions.

Spatial regulation of the expression of the gap genes, the first zygotic patterning genes to be expressed during embryogenesis, is determined by the activity of the three

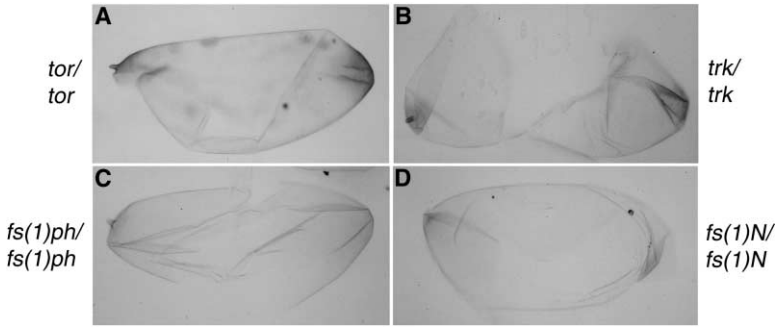

Figure 2. Polar Localization of Tsl Is Disrupted in Eggs from *fs(1)ph* and *fs(1)N* Mutant Mothers

(A–D) Bright-field images of VMs stained with antibodies against Tsl. Maternal genotypes are shown. Polar localization of Tsl is not affected by maternal mutations in (A) *tor* or (B) *trk*. Maternal mutations in (C) *fs(1)ph* and (D) *fs(1)N* result in a broader distribution of Tsl protein over the poles at apparently reduced concentrations relative to wild-type.

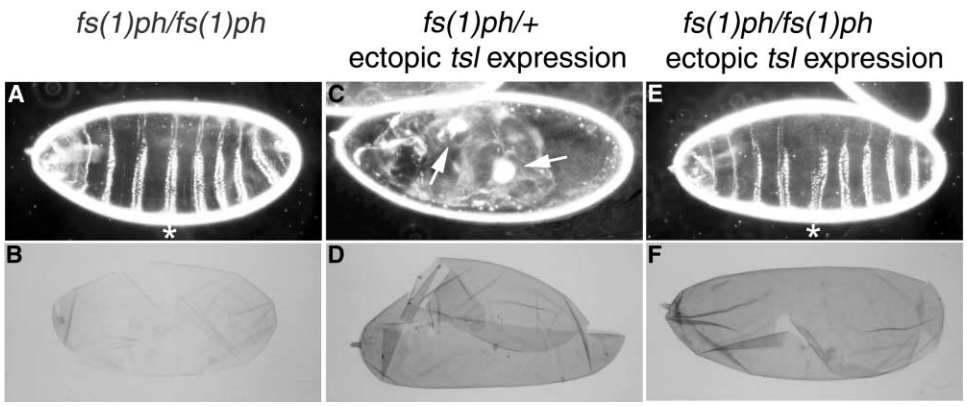

Figure 3. *fs(1)ph* Is Required for Tsl Function, but Not Its Association with the VM

(A–F) Dark-field images of (A, C, and E) embryonic cuticles and bright-field images of (B, D, and F) VMs stained with antibodies against Tsl. Maternal genotypes are shown. (A) An embryo derived from an *fs(1)ph* mutant mother lacks all structures posterior to abdominal segment 8, and the head skeleton is reduced in size. The segmented thorax and abdomen are characterized by the presence of ventral denticle bands (indicated by an asterisk). (B) Polar localization of Tsl to the VM is disrupted in *fs(1)ph* maternally mutant embryos. (C) An embryo derived from an *fs(1)ph/+* heterozygous mother expressing ectopic *tsl* throughout the follicle cell layer exhibits a gain-of-function phenotype in which the segmented region is deleted but terminally derived Filzkörper material (arrows) is present. (D) The concentration of Tsl on the VM of these embryos is uniformly high. (E) An embryo from an *fs(1)ph/fs(1)ph* homozygous mutant mother ectopically expressing *tsl* exhibits the *fs(1)ph* mutant phenotype, despite high levels of Tsl on the VM (F).

maternal pathways (anterior, posterior, and terminal) required for the development of the anterior-posterior axis of the embryo [20]. In addition to localized maternal input, interactions between the gap gene products themselves lead to further refinement of their expression domains [21]. *tll* expression, for example, is specifically repressed in the segmented region of the embryo by central gap gene products such as Kr [22]. Thus, depending on their relative levels of activity, Kr and Tll are both capable of suppressing one another's expression. This raised the possibility that centrally expressed Kr was responsible for the polar restriction of *tll* expression that we observed in the progeny of *tsl* mutant females expressing *tsl* from the germline. To address this question, we crossed the *CBBtsl* insertion into females that were mutant for all three anterior-posterior maternal pathways. Embryos produced by mothers triply mutant for *bicoid* (anterior), *oskar* (posterior), and *tsl* (terminal) lack all anterior-posterior patterning [22, 23] (Figure 5A), express low levels of *Kr* uniformly along the anterior-posterior axis [22, 23] (Figure 5E), and do not express *tll* at all [23] (Figure 5C). In contrast, the embryos pro-

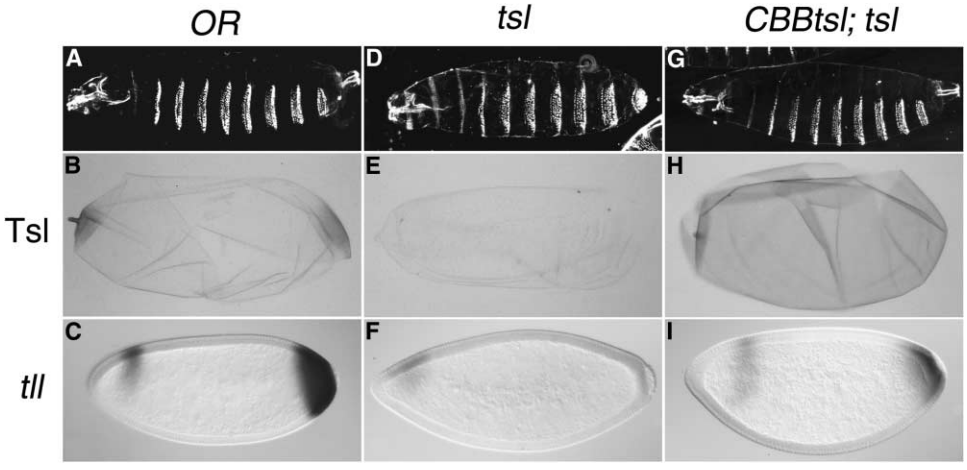

Figure 4. Germline-Expressed *tsl* Restores Polar *tll* Expression and Cuticular Structures in Embryos Maternally Mutant for *tsl*

(A–I) (A, D, and G) Cuticles of developed embryos, (B, E, and H) VMs stained for Tsl, and (C, F, and I) cellular blastoderm embryos hybridized with a probe for *tll*. Maternal genotypes shown above. (A) Wild-type cuticle with normal head, segmented region, and tail. (B) Wild-type VM displays two polar domains of Tsl. (C) In wild-type embryos, *tll* is expressed in a conspicuous cap at the posterior and a dorsal stripe at the anterior. (D) Embryonic cuticle showing the *tsl* loss-of-function phenotype-reduced head skeleton and the absence of structures that normally develop posterior to A7. (E) The VM of an egg derived from a $tsl^{PZRev32}$ mutant mother lacks Tsl staining, and there is no posterior *tll* expression in the embryos (F). Residual anterior expression is due to positive input from the *bicoid* gene [35]. (G) An embryo from a *tsl* mutant mother expressing *tsl* in the germline exhibits complete restoration of terminal cuticular structures. (H) Germline expression of *tsl* leads to uniform distribution of Tsl protein on the VM, which restores polar *tll* expression (I).

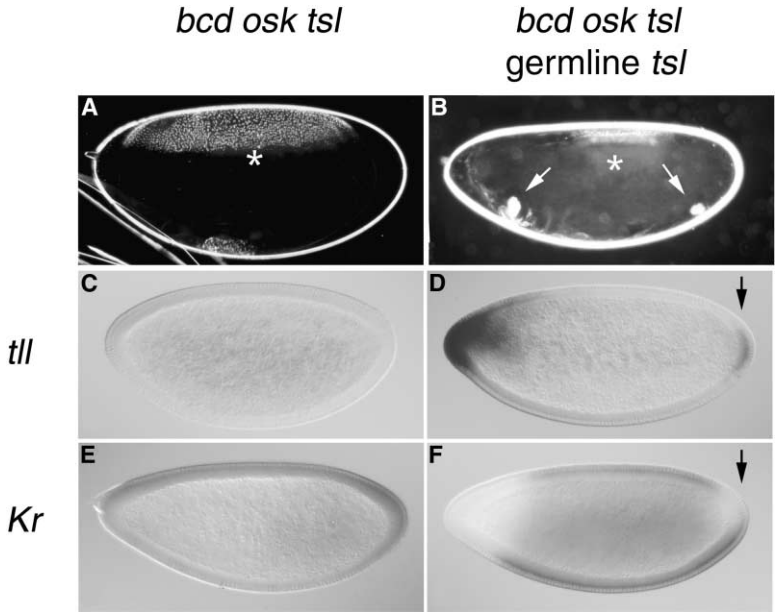

Figure 5. Germline-Expressed *tsl* Polarizes Embryos Lacking Maternal Anterior-Posterior Patterning Information

Maternal genotypes are shown above the figure.

(A and B) Cuticle preparations of developed embryos.

(C–F) Cellular blastoderm embryos hybridized with a probe for (C and D) *tll* or (E and F) *Kr*. (A) In the cuticle of an embryo from a *bcd osk tsl* mutant mother, ventral denticles (indicated by an asterisk) are present all along the anterior-posterior axis, which displays no anterior-posterior polarity. No terminal cuticular structures are present. (B) In the cuticle of an embryo derived from a *bcd osk tsl* mutant mother expressing *tsl* in the germline, Filzkörper material (arrows) differentiated at the poles, and ventral denticles (indicated by an asterisk) were restricted to the central region of the embryo. (C) An embryo derived from a *bcd osk tsl* mutant mother does not express *tll*. (D) An embryo from a *bcd osk tsl* mother expressing *tsl* in the germline exhibits *tll* expression in a large anterior cap and a smaller domain at the posterior pole (arrow). The expression of *Kr* is complementary to that of *tll*. *Kr* is uniformly expressed in the progeny of *bcd osk tsl* mutant mothers (E), and it is repressed at the anterior and posterior (arrow) poles of embryos from *bcd osk tsl* mutant mothers expressing *tsl* in the germline (F). The apparent dorsal-ventral asymmetry in staining intensity seen in (E) and (F) is due to shadows cast by the DIC optics used to visualize the staining.

duced by triply mutant females carrying *CBBtsl* did express *tll* in distinct polar domains, either at the anterior alone or at both poles (Figure 5D). Consistent with this pattern of *tll* expression, *Kr* expression was specifically repressed in the corresponding polar domains (Figure 5F). Further, these embryos also differentiated Filzkörper material, a posterior cuticular structure that requires terminal pathway activity, at one or both poles (Figure 5B). Thus, although Tsl was distributed uniformly in their VMs, these embryos developed gap gene expression patterns and cuticular phenotypes consistent with polar activation of the Tor receptor. These findings suggest that the activity of Tsl is enhanced at, and perhaps restricted to, the polar regions of the VM; this finding implies that there is an as yet unidentified component of the terminal class pathway that is restricted to the poles and is required for the function of Tsl.

The expression of *tsl* by the somatic follicle cells, and its role in patterning the embryo, can be thought of as an inductive event between the soma and the germline. However, the delay between the secretion of Tsl during oogenesis and the activation of the Tor receptor during embryogenesis necessitates stabilization of the localized signal. We have shown that this is achieved by the incorporation of Tsl into the eggshell. The localization of Tsl on the inside of the VM allows it to be accessible to components of the perivitelline fluid, such as the Trk precursor, and the restriction of its activity to the poles of the VM limits the spatial parameters of Tor activation. To our knowledge, this is the first demonstration of an embryonic patterning molecule associated with the eggshell.

The development of the dorsal-ventral axis in *Drosophila* embryos also requires the transfer of patterning information from the soma to the germline [24], which leads to the asymmetric activation of a uniformly distributed receptor by a locally processed ligand that shares structural elements with the Trk ligand [3, 25]. It is intriguing to note that, like *fs(1)ph* and *fs(1)N*, one of the genes required for dorsal-ventral development, *nudel*, is required for the formation of the eggshell [26]. Thus, our findings raise the possibility that spatial information for dorsal-ventral patterning may also be stored in the VM. However, none of the known genes in this pathway share homology with Tsl, which carries structural features, such as a membrane-attack complex/perforin domain [27], that may promote membrane interactions. Although the VM is not a classic lipid membrane, it is highly hydrophobic. Our data indicate that even in embryos maternally mutant for $fs(1)ph^{1901}$ or $fs(1)N^{211}$, Tsl protein is still associated with the VM; this association demonstrates that Tsl has an affinity for the VM that is independent of its interaction with Polehole and Nasrat. An intriguing possibility is that Polehole and Nasrat are required to stabilize secreted Tsl such that it becomes incorporated into the VM in an active conformation.

The existence of another localized factor in this pathway indicates that there are at least four levels of control that ensure the polar restriction of *tll* expression during embryonic development. First to act is the restriction of *tsl* expression to a specific subpopulation of follicle cells present at the poles of the oocyte. Second is the stabilization of secreted Tsl protein at the poles of the VM and its incorporation into the eggshell in an active form. Third is the facilitation of Tsl function through its proposed interaction with another localized factor. The final layer of control is the exclusion of *tll* expression from nonpolar regions through the inhibitory effects of centrally expressed gap genes. Although it has long been assumed that the spatial restriction of *tsl* expression was the

uniquely localized element in the terminal pathway, we present data here implying the existence of another factor that enhances the activity of Tsl specifically at the poles. The function of Tsl itself is unknown, and there are currently no candidate genes encoding proteins with the enzymatic activity to bring about the proposed processing of the Trk precursor. It is likely, therefore, that the identification of this factor will greatly enhance our understanding of the mechanism by which the Tor ligand is formed.

Experimental Procedures

Fly Stocks and Maintenance

All mothers and developing embryos were maintained at 26.5°C. To view embryonic cuticles, embryos were collected overnight from females of the appropriate genotype, allowed to complete embryogenesis, and then prepared according to standard methods [28]. The wild-type stock was Oregon R. The following mutations are described as referenced: *bcd*^{6}, *osk*^{6}, and *trk*^{4} (Flybase [http://www.flybase.bio.indiana.edu]), tor^{XR1} [1], and *fs(1)ph*^{1901} and *fs(1)N*^{211} [7–9]. The *tsl*^{PZRev32} allele was generated from the *tsl*^{PZ} enhancer trap line [5] through imprecise excision. It behaves genetically as a null mutation. Southern blot and DNA sequence analysis indicated that this mutation carries a 3 kb deletion of the 5′ untranslated region (data not shown) that includes the presumed transcriptional start site [5].

Antibody Staining

Antisera against Tsl was generated in rabbits that were injected with a histidine-tagged fusion protein (Novagen) containing the C-terminal two-thirds of the Tsl protein. Antibodies against Tsl were affinity-purified on a column containing the immunizing protein coupled to Affigel (Biorad). Potential cross-reacting antibodies were removed by incubation with Sepharose gel coupled to a homogenate made from female flies homozygous for *tsl*^{PZRev32}, followed by preabsorption against fixed embryos from *tsl*^{PZRev32} females. Collected embryos were dechorionated in 50% bleach, washed, and then attached to a glass microsope slide coated with a thin layer of glue (made by extracting packing tape with heptane). The slides were then immersed in phosphate-buffered saline (PBS), and the embryos were removed from their VMs by pricking the VMs with a 26G needle and then pushing the embryos out of the opening. The empty VMs were then incubated in 4% paraformaldehyde for 20 min and were subsequently rinsed with PBS. The empty VMs were then processed for antibody staining according to Han et al. [29].

Directed Expression of *tsl*

The *Gal4#3* insertion has been described [30]. To place *tsl* under Gal4 transcriptional control, we cloned the *tsl* cDNA downstream of the UAS sequences in the pUAST vector [13] by using the EcoR1 cloning site. This construct, UAStsl, was transformed into flies by using standard methods [28]. To express *tsl* from the germline, we used in vitro mutagenesis to introduce an NcoI site at the starting ATG in the *tsl* cDNA [5]. We then cloned the NcoI-EcoR1 fragment containing the entire *tsl* coding region and the 3′ flanking region into pSPBP4, which contains a modified *Xenopus* β-globin leader to enhance translation [31]. Plasmid BP4tsl was digested with HindIII and EcoR1, releasing a fragment carrying *tsl* downstream of the β-globin leader. This fragment was made blunt ended by treating with Klenow enzyme, and it was then ligated to BclI linkers. Following BclI digestion, the *tsl* fragment was ligated to BglII-digested pCasperbcdBglII (CBB) [32], a P element-based transformation vector containing 5′ and 3′ untranslated sequences from the *bcd* gene. This construct, termed CBBtsl, was transformed into flies by using conventional injection techniques [28]. It should be noted that the *bcd* 3′ region contained within the CBB vector lacks sequences that are required for the anterior localization of *bcd* RNA in the embryo [33]. In addition, the *bcd* 5′ sequences that were utilized in the construction of the CBB vector lack elements that are required to direct high levels of *bcd* transcription [33]. Thus, maternal expression of *tsl* from this construct would be expected to generate low levels of uniformly distributed *tsl* mRNA in the embryo; we confirmed this expectation through in situ hybridization (data not shown).

In Situ Hybridization

In situ hybridization to embryos was carried out as described [34], with the following modifications: 10% dimethylsulfoxide was added to the paraformaldehyde fixative, and the incubation with anti-digoxigenin antibody was carried out overnight at 4°C at a concentration of 1:5,000, followed by a 30–60 min incubation at room temperature. Hybridization probes were prepared in a random priming reaction by using full-length cDNAs as templates.

Acknowledgments

We thank Denise Montell for providing the *tsl* cDNA and the *tsl*^{PZ} strain of flies, Jennifer Choe for help in isolating the *tsl*^{PZRev32} line, Andrea Brand and Norbert Perrimon for providing the pUAST plasmid and flies carrying the enhancer detector Gal4 vector, pGawB, Stephen Small for the *Kr* cDNA, Charles Smith for fly food preparation, Iris Koch and Christiane Nüsslein-Volhard for providing flies from the Tübingen Stock Collection, and Martin Klingler for discussion. This work was supported by grants from the National Institutes of Health to L.M.S. and D.S.

Received: March 24, 2003
Revised: April 28, 2003
Accepted: April 28, 2003
Published: June 17, 2003

References

1. Sprenger, F., Stevens, L.M., and Nüsslein-Volhard, C. (1989). The *Drosophila* gene *torso* encodes a putative receptor tyrosine kinase. Nature *338*, 478–483.
2. Casanova, J., and Struhl, G. (1989). Localized surface activity of *torso*, a receptor tyrosine kinase, specifies terminal body pattern in *Drosophila*. Genes Dev. *3*, 2025–2038.
3. Casanova, J., Furriols, M., McCormick, C.A., and Struhl, G. (1995). Similarities between *trunk* and *spätzle*, putative extracellular ligands specifying body pattern in *Drosophila*. Genes Dev. *9*, 2539–2544.
4. Casali, A., and Casanova, J. (2001). The spatial control of Torso RTK activation: a C-terminal fragment of the Trunk protein acts as a signal for Torso receptor in the *Drosophila* embryo. Development *128*, 1709–1715.
5. Savant-Bhonsale, S., and Montell, D.J. (1993). *torso-like* encodes the localized determinant of *Drosophila* terminal pattern formation. Genes Dev. *7*, 2548–2555.
6. Martin, J.-R., Raibaud, A., and Ollo, R. (1994). Terminal pattern elements in *Drosophila* embryo induced by the *torso-like* protein. Nature *367*, 741–745.
7. Perrimon, N., Mohler, D., Engstrom, L., and Mahowald, A.P. (1986). X-linked female-sterile loci in *Drosophila melanogaster*. Genetics *113*, 695–712.
8. Degelmann, A., Hardy, P.A., Perrimon, N., and Mahowald, A.P. (1986). Developmental analysis of the Torso-like phenotype in *Drosophila* produced by a maternal-effect locus. Dev. Biol. *115*, 479–489.
9. Degelmann, A., Hardy, P.A., and Mahowald, A.P. (1990). Genetic analysis of two female-sterile loci affecting eggshell integrity and embryonic pattern formation in *Drosophila melanogaster*. Genetics *126*, 427–434.
10. Cernilogar, F.M., Fabbri, F., Andrenacci, D., Taddei, C., and Gargiulo, G. (2001). *Drosophila* vitelline membrane cross-linking requires the *fs(1)Nasrat*, *fs(1)polehole* and chorion genes activities. Dev. Genes Evol. *211*, 573–580.
11. Jiménez, G., González-Reyes, A., and Casanova, J. (2002). Cell surface proteins Nasrat and Polehole stabilize the Torso-like extracellular determinant in *Drosophila* oogenesis. Genes Dev. *16*, 913–918.
12. Furriols, M., Casali, A., and Casanova, J. (1998). Dissecting the

mechanism of Torso receptor activation. Mech. Dev. *70*, 111–118.
13. Brand, A., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.
14. Sprenger, F., and Nüsslein-Volhard, C. (1992). Torso receptor activity is regulated by a diffusable ligand produced at the extracellular terminal regions of the *Drosophila* egg. Cell *71*, 987–1001.
15. Casanova, J., and Struhl, G. (1993). The *torso* receptor localizes as well as transduces the spatial signal specifying terminal body pattern in *Drosophila*. Nature *362*, 152–155.
16. Klingler, M., Erdélyi, M., Szabad, J., and Nüsslein-Volhard, C. (1988). Function of *torso* in determining the terminal anlagen of the *Drosophila* embryo. Nature *335*, 275–277.
17. Strecker, T.R., Halsell, S.R., Fisher, W.W., and Lipshitz, H.D. (1989). Reciprocal effects of hyper- and hypoactivity mutations in the *Drosophila* pattern gene *torso*. Science *243*, 1062–1066.
18. Pignoni, F., Baldarelli, R.M., Steingrímmson, E., Diaz, R.J., Patapoutian, A., Merriam, J.R., and Lengyel, J.A. (1990). The *Drosophila* gene *tailless* is expressed at the embryonic termini and is a member of the steroid receptor superfamily. Cell *62*, 151–163.
19. Steingrímsson, E., Pignoni, F., Liaw, G.-J., and Lengyel, J.A. (1991). Dual role of the *Drosophila* pattern gene *tailless* in embryonic termini. Science *254*, 418–421.
20. St. Johnston, D., and Nüsslein-Volhard, C. (1992). The origin of pattern and polarity in the *Drosophila* embryo. Cell *68*, 210–219.
21. Rivera-Pomar, R., and Jäckle, H. (1996). From gradients to stripes in *Drosophila* embryogenesis: filling in the gaps. Trends Genet. *12*, 478–483.
22. Greenwood, S., and Struhl, G. (1997). Different levels of Ras activity can specify distinct transcriptional and morphological consequences in early *Drosophila* embryos. Development *124*, 4879–4886.
23. Struhl, G., Johnston, P., and Lawrence, P.A. (1992). Control of *Drosophila* body pattern by the Hunchback morphogen gradient. Cell *69*, 237–249.
24. Morisato, D., and Anderson, K.V. (1995). Signaling pathways that establish the dorsal-ventral pattern of the *Drosophila* embryo. Annu. Rev. Genet. *29*, 371–399.
25. DeLotto, Y., and DeLotto, R. (1998). Proteolytic processing of the *Drosophila* Spätzle protein by Easter generates a dimeric NGF-like molecule with ventralising activity. Mech. Dev. *72*, 141–148.
26. LeMosy, E.K., and Hashimoto, C. (2000). The Nudel protease of *Drosophila* is required for eggshell biogenesis in addition to embryonic patterning. Dev. Biol. *217*, 352–361.
27. Ponting, C.P. (1999). Chlamydial homologues of the MACPF (MAC/perforin) domain. Curr. Biol. *9*, R911–R913.
28. Roberts, D.B. (1986). *Drosophila*, A Practical Approach. (Oxford: IRL Press).
29. Han, D.D., Stein, D., and Stevens, L.M. (2000). Investigating the function of follicular subpopulations during *Drosophila* oogenesis through hormone-dependent enhancer-targeted cell ablation. Development *127*, 573–583.
30. Sen, J., Goltz, J.S., Stevens, L., and Stein, D. (1998). Spatially restricted expression of *pipe* in the *Drosophila* egg chamber defines embryonic dorsal-ventral polarity. Cell *95*, 471–481.
31. Siegel, V., and Walter, P. (1988). Each of the activities of signal recognition particle (SRP) is contained within a distinct domain: analysis of biochemical mutants of SRP. Cell *52*, 39–49.
32. Stein, D., Goltz, J.S., Jurcsak, J., and Stevens, L. (1998). The Dorsal-Related Immunity factor (Dif) can define the dorsal-ventral axis of polarity in the *Drosophila* embryo. Development *125*, 2159–2169.
33. Macdonald, P.M., and Struhl, G. (1988). *Cis*-acting sequences responsible for anterior localization of *bicoid* mRNA in *Drosophila* embryos. Nature *336*, 595–598.
34. Tautz, D., and Pfeifle, C. (1989). A non-radioactive *in situ* hybridization method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmentation gene *hunchback*. Chromosoma *98*, 81–85.
35. Pignoni, F., Steingrímsson, E., and Lengyel, J.A. (1992). *bicoid* and the terminal system activate *tailless* expression in the early *Drosophila* embryo. Development *115*, 239–251.