

Jeremy A. Lynch · Eugenia C. Olesnický ·
Claude Desplan

Regulation and function of *tailless* in the long germ wasp *Nasonia vitripennis*

Received: 14 January 2006 / Accepted: 4 April 2006 / Published online: 3 May 2006
© Springer-Verlag 2006

Abstract In the long germ insect *Drosophila*, the gene *tailless* acts to pattern the terminal regions of the embryo. Loss of function of this gene results in the deletion of the anterior and posterior terminal structures and the eighth abdominal segment. *Drosophila tailless* is activated by the maternal terminal system through Torso signaling at both poles of the embryo, with additional activation by Bicoid at the anterior. Here, we describe the expression and function of *tailless* in a long germ Hymenoptera, the wasp *Nasonia vitripennis*. Despite the morphological similarities in the mode of development of these two insects, we find major differences in the regulation and function of *tailless* between *Nasonia* and *Drosophila*. In contrast to the fly, *Nasonia tll* appears to rely on *otd* for its activation at both poles. In addition, the anterior domain of *Nasonia tll* appears to have little or no segmental patterning function, while the posterior *tll* domain has a much more extensive patterning role than its *Drosophila* counterpart.

Keywords *Nasonia* · *Tailless* · Terminal system · Orthodenticle · Evolution of development

Introduction

In *Drosophila*, three maternal coordinate systems combine to pattern the anterior–posterior axis of the embryo. The anterior system patterns the head and thorax, the posterior patterns the abdomen, and the terminal pathway acts to pattern the extreme anterior and posterior nonsegmented regions of the embryo (Duffy and Perrimon 1994;

Nusslein-Volhard 1991). The terminal system relies on localized signaling through the receptor tyrosine kinase Torso (Duffy and Perrimon 1994). Both Torso receptor and its putative ligand, Trunk, are ubiquitously expressed throughout the oocyte, and it appears that localized Torso activation results from processing and activation of *trunk* by *torso-like*, whose expression is restricted to ovarian follicle cells overlying the anterior and posterior poles of the oocyte (Casali and Casanova 2001; Savant-Bhonsale and Montell 1993; Stevens et al. 1990).

Activation of Torso, and subsequently of the Ras pathway, leads to the localized derepression of two zygotic genes that mediate most, if not all, maternal terminal gene function, *tailless* (*tll*) and *huckebein* (*hkb*) (Weigel et al. 1990), with the cap of *hkb* expression being more terminal than the stripe of *tll* expression. *tll* encodes an orphan nuclear receptor. This gene is initially expressed in symmetrical caps at both poles in response to *torso* signaling (Pignoni et al. 1990; Weigel et al. 1990). Mutations in *tll* result in the loss of the anterior and posterior terminal structures and also of the eighth abdominal segment (Mahoney and Lengyel 1987). *tll* was shown to be required to activate specific posterior target genes, including the posterior domain of the gap gene *hunchback* (*hb*) (Margolis et al. 1995). It also appears to have a role in setting borders of gap and pair-rule genes in the head and trunk of the embryo, although these effects have not been shown to be direct (Weigel et al. 1990). Later, the anterior cap domain of *tll* expression is replaced by a stripe that is under control of the Bicoid gradient. This domain appears to be involved in patterning the most anterior region of the fly brain (Pignoni et al. 1992).

Tailless protein (Tll) has two highly conserved domains: a zinc finger DNA-binding domain at the N terminus and a putative ligand-binding domain at the C terminus. *tll* orthologs have been found in many organisms outside of the fly, including deuterostomes. In vertebrates, *tlx* genes are involved in patterning the anterior of the brain and eyes. Because these functions are also conserved with *Drosophila tll*, they may represent the ancestral functions for this gene among metazoans (Yu et al. 1994).

Communicated by guest editors Jean Deutsch and Gerhard Scholtz

J. A. Lynch · E. C. Olesnický · C. Desplan (✉)
Center for Developmental genetics, Department of Biology,
New York University,
1009 Silver Center, 100 Washington Square East,
New York, NY 10003, USA
e-mail: cd38@nyu.edu
Tel.: +1-212-9988218
Fax: +1-212-9954710

Analyses of *tll* expression and function in other insects have provided evidence that the role of this gene in patterning the axis can differ substantially in different species. The role of *tll* has been examined in the short germ embryo of the beetle *Tribolium castaneum* (Coleoptera). As a short germ embryo, the *Tribolium* fate map differs significantly from that of *Drosophila*: Whereas the anterior region of the egg gives rise to the anterior of the embryo in *Drosophila*, in *Tribolium*, it becomes extraembryonic membranes, with the embryonic anlage restricted toward the posterior of the egg. In addition, the posterior segments are not patterned at the blastoderm stage, as they are in *Drosophila*, but rather arise later in a posterior growth zone (Davis and Patel 2002). Despite these differences, *torso* signaling was shown to be active at both poles of the *Tribolium* egg, similar to *Drosophila*, but the patterning function of these domains is different, due to the differing fate maps of these two embryos. *torso* signaling at the anterior affects the specification of the extraembryonic membranes, while in the posterior, it is involved in the extension of the germband (Schoppmeier and Schroder 2005). These differences are reflected by the expression of *torso* targets, particularly *tll*.

In the early blastoderm stage, *Tribolium tailless* expression is seen only at the posterior. Later, just before gastrulation, *tll* expression is seen at the anterior of the presumptive embryo (which lies at this time in the middle of the egg, far from the anterior pole) and disappears from the posterior (Schroder et al. 2000). Knocking down either *torso* or *torso-like* function results in the loss of the posterior domain of *tailless* (Schoppmeier and Schroder 2005), indicating conservation in the regulation of the posterior *tailless* domains of beetles and flies. However, consistent with its late appearance, the anterior domain is likely not under terminal maternal control, and might be equivalent to the brain patterning function of *tll* in *Drosophila* and vertebrates.

The expression of *tll* was also examined in the mosquito *Anopheles*, another Diptera, which undergoes long germ embryogenesis. In this organism, *tll* is expressed in anterior and posterior cap domains, which appear to be more extensive than those seen in *Drosophila*, possibly indicating a broader patterning role in the mosquito (Goltsev et al. 2004). However, no functional studies have been reported.

In this paper, we report expression, regulation, and functional data on the *tll* ortholog from the wasp *Nasonia vitripennis*. The *Nasonia* embryo undergoes long germ embryogenesis that is similar to that of *Drosophila*, but these two organisms belong to two distantly related groups (Hymenoptera and Diptera). In fact, it has been proposed that the long germ mode of embryogenesis in the wasp is independently derived from that of flies (Lynch et al. 2006; Savard et al. 2006, submitted for publication). We have found that, while some aspects of *tll* expression and function appear to be conserved with those in *Drosophila*, there are many significant differences as well.

Materials and methods

Cloning *Nasonia tll*

A fragment of the *N. vitripennis tailless* gene was cloned using degenerate PCR with primers designed using the CODEHOP strategy (<http://blocks.fhcr.org>). The primers were from the zinc-finger coding region and had the following sequences: forward-GCACTACGGGATCTAC GCCTGYGAYGGNTG and reverse-CGCTCGTGCTGC ACCGCRCTCYTTRTTCA. This sequence was extended in the 3' direction using the SMART RACE kit from Clontech, following the manufacturer's instructions. This sequence has been submitted to Genbank under accession number DQ324544.

Embryo collection and in situ hybridization

Wasps were incubated with *Sarcophaga bullata* hosts for 3 h at 28°C, after which hosts were removed from wasps and were aged for an additional 3 h. Embryos were then collected from hosts fixed in 10% formaldehyde under heptane for 20 min with vigorous shaking. The embryos were affixed with double-sided tape, and vitelline membranes were removed under phosphate-buffered saline using 28 1/2 gauge needles. In situ hybridization was carried out using standard protocols.

First instar larval cuticle preparation

Embryos from *tll* dsRNA injected mothers were allowed to age until cuticle structures were visible. These were then mounted in 90% lactic acid, 10% ethanol, and incubated overnight at 65°C. Cleared cuticles were observed and photographed using dark field illumination.

Parental RNAi

A PCR product, produced using hybrid primers with *Nasonia tll* specific sequence at the 3' ends and T7 RNA polymerase binding sites at the 5' ends, was used as a template for double-stranded RNA production using the Ambion MEGASCRIPT kit. *otd* dsRNA production is described elsewhere (Lynch et al. 2006). Early (yellow with no eye pigment) *Nasonia* pupae were injected with dsRNA at a concentration of approximately 1 mg/ml. While the apparatus used to inject the pupae (a pulled glass capillary needle attached to a 10-ml syringe by a length of tubing) does not lend itself to accurate estimation of the volume delivered, consistent results among wasps was achieved by observing visible swelling of the pupae while injecting.

Results and discussion

Embryonic expression of *Nasonia tailless*

A degenerate PCR strategy was employed to clone the *Nasonia tll* ortholog. Primers were designed based on the highly conserved zinc finger DNA-binding domain. One PCR product was obtained. The sequence was extended by using 3' RACE. The resulting sequence contained both conserved domains of *tll* orthologs, the zinc finger, and the putative ligand-binding domain, which indicates that this sequence corresponds to the *Nasonia tll* ortholog.

Nasonia tll expression was first detected in the syncytial blastoderm stage of embryogenesis. No maternal expression was observed in earlier embryos or ovaries (data not shown). Initially, *tll* expression was seen as a stripe across the anterior pole of the embryo and as a broad posterior cap (Fig. 1a). Later in the cellular blastoderm stage, the anterior domain split medially and dorsally, and formed two triangular domains covering the dorsal anterior regions on either side of the dorsal midline (Fig. 1b). The dynamic expression of this domain is quite reminiscent of the *bcd*-dependent anterior *tll* stripe in *Drosophila*. Just before gastrulation, the posterior cap resolved into a narrow posterior stripe (Fig. 1c).

It is thus clear that at least one aspect of *tll* regulation at the anterior is different in *Nasonia* as compared to

Drosophila: In the fly, the initial expression of *tll* is in a fairly broad anterior cap in response to *torso* signaling, which then evolves into a *bcd*-dependent anterior stripe. In *Nasonia*, no anterior cap was seen, indicating a possible change in regulation of the anterior *tll* domain.

Regulation of *Nasonia tailless*

While in *Drosophila*, *tll* expression in symmetric caps responds to terminal Torso signaling at both poles, only the posterior aspect of expression responds to this system in *Tribolium* (Schoppmeier and Schroder 2005). In *Nasonia*, no components of the terminal signaling system have yet been examined, and it is not even clear whether the Torso system is required for *tll* expression in this organism because *tll* expression is not seen until late into the syncytial blastoderm stage. In addition, the anterior domain of *Nasonia tll* is not expressed in a cap, as might be expected if it were being activated by a gradient of terminal signaling in a manner similar to that seen in *Drosophila*. Instead, the anterior domain is expressed in a stripe that is reminiscent of the *Drosophila* late *bcd*-dependent stripe expression, rather than the early terminal system dependent cap.

While *Nasonia* lacks a *bcd* ortholog, we have shown that the gene *orthodenticle-1* (*otd1*) plays a *bcd*-like role in this organism (the *Nasonia orthodenticle2* gene is expressed only zygotically and likely has a limited role in early embryonic patterning; unpublished observation). In addition, we also showed that *otd1* also plays a morphogenetic role in posterior patterning (Lynch et al. 2006). We tested the role of *otd1* in activating *tll* expression by knocking down its expression using parental RNAi. When *otd1* was reduced in this manner, a progressive reduction of *tll* expression at both ends of the embryo was observed (Fig. 2). The variation in *tll* expression levels likely depends on the severity of *otd1* knockdown. These results show that *otd1* function is required for both expression domains of *tll* expression in *Nasonia*, while it depends on Torso signaling at both poles in *Drosophila*. Therefore, *Nasonia tll* expression might not require activation of the terminal pathway and might instead depend entirely on *otd1*.

This result suggests that the terminal system dependent anterior cap expression of *tll* in *Drosophila* is a novel feature that arose along the lineage leading to the Diptera after the split from the Hymenoptera and Coleoptera, as this pattern is not seen in the wasp *Nasonia* or the beetle *Tribolium* (Schroder et al. 2000). Instead, at least within the holometabola, the anterior *tll* may have ancestrally been regulated by the anterior patterning system, rather than the terminal system.

At the posterior, *Nasonia tll* is expressed in a broad cap, which is similar to what is seen in both *Tribolium* and *Drosophila*. However, this domain is dependent on *otd1* in *Nasonia*, which is quite different from what is seen in the two other insect species, where *otd* is unlikely to have any posterior patterning role. This result shows that the

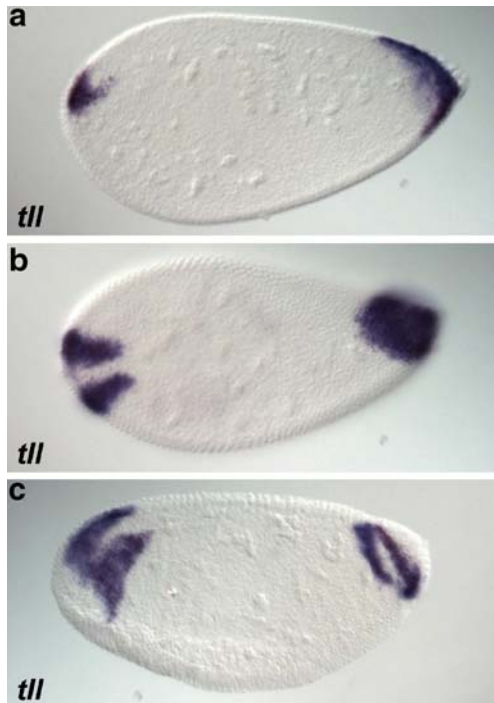


Fig. 1 Expression of *tailless* in the *Nasonia* embryo. During the syncytial blastoderm stage, *tll* expression is first seen as a stripe across the anterior pole of the embryo (in this lateral view this stripe appears as a spot), and in a broad posterior cap (a) (lateral view). Later, the anterior stripe splits along the dorsal midline, and forms two symmetrical triangular domains on either side of the midline (b) (Dorsal view). Just before gastrulation, the posterior cap has resolved into a narrow stripe, while the anterior triangular domains appear to broaden (c) (lateral view)

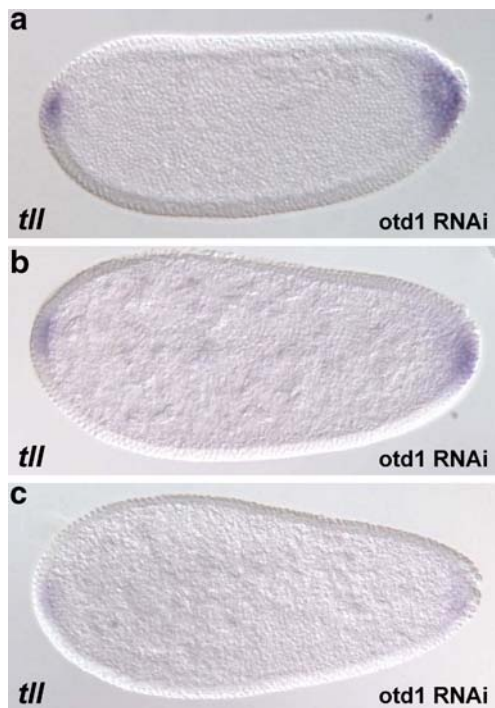


Fig. 2 *otd* is required for both the anterior and posterior expression domains of *Nasonia tll*. The amount of knockdown of a particular gene has been shown to vary significantly among different embryos. Here, we show the effect of *otd1* RNAi on *tll* expression: **a** weakly affected (4/22, 18%), **b** moderately affected (11/22, 50%), **c** strongly affected (7/22, 32%). The degree to which *tll* expression is reduced is likely to be correlated with the degree to which *otd1* function has been reduced, which was shown to be variable (Lynch et al. 2006)

regulation of patterning genes can adapt to novel features of embryonic patterning environments and that the terminal system might be dispensable in *Nasonia*, at least for *tll* expression. It will become important to test whether there is a *hkb*-like gene in *Nasonia*, and whether it also depends on *otd1* or on a terminal system. A number of components of the terminal system have been identified in *Nasonia*, including potential orthologs of *torso-like*, *trunk*, and *torso*, and it will be of interest to understand the role of these genes in this organism.

Anterior embryonic patterning function of *Nasonia tailless*

In *Drosophila*, *tll* is primarily involved in patterning the anterior and posterior terminal regions, as well as posterior gut primordia. (Mahoney and Lengyel 1987). The role of *tll* in patterning the *Nasonia* embryo was tested by parental RNAi (Bucher et al. 2002). No defects were apparent at the anterior when first instar larval cuticles of the resulting embryos were examined (Fig. 3a,b). Because there are no clear anterior terminal structures on the *Nasonia* cuticle, the most anterior structure is the labrum, which is unaffected (data not shown). Thus it appears that the anterior *tll* domain does not play an important role in patterning anterior cuticle structures, and the function of this domain may be restricted to patterning the anterior nervous system of the wasp.

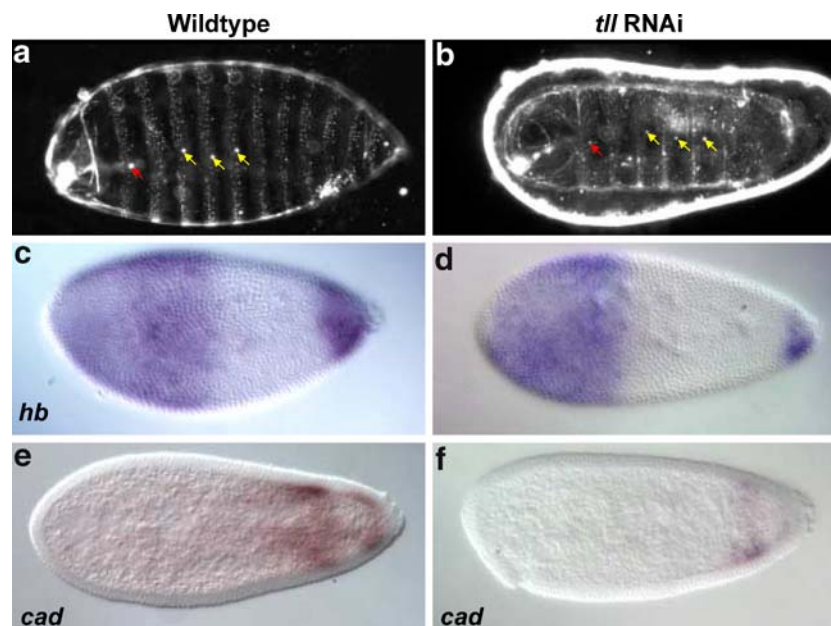


Fig. 3 *Nasonia tll* is required to pattern a large posterior portion of the embryo and is needed for proper activation of potential target genes. **a** Wild-type first instar larval cuticle. The red arrow points to the spiracle marking the second thoracic segment, while the yellow arrows point to spiracles that mark the first, second, and third abdominal segments. **b** A severely affected cuticle from a *tll* dsRNA injected mother. Only one disorganized denticle belt is seen posterior to the third abdominal segment, indicating a very broad

posterior patterning role for *tll*. The anterior appears to be unaffected. **c** Wild-type *hunchback* expression. **d** The posterior domain of *hb* is significantly reduced in *tll* RNAi embryos. **e** Wild-type *caudal* expression in the late blastoderm stage. **f** *cad* expression at similar stage to **e** in a *tll* RNAi embryo, showing a reduction of the most posterior stripe, and a posterior shift for the more anterior stripe

This limited role for *Nasonia tll* in anterior axial patterning is also likely to be shared in *Tribolium*, where anterior *tll* is expressed late in a very restricted domain that is unlikely to have a significant effect on any downstream axis patterning genes (Schroder et al. 2000). Thus, it appears that the axis patterning role for anterior *tll* is correlated with it coming under the regulation of the terminal system along the *Drosophila* lineage. This novel mode of regulation may have been permissive for the acquisition of new patterning functions by allowing expression earlier in development and in a broader domain.

Posterior embryonic patterning function of *Nasonia tailless*

In contrast to the apparent lack of axial patterning function of the anterior domain, *Nasonia tll* appears to have a broad posterior patterning role. In the most severe *tll* pRNAi phenotypes, up to five posterior abdominal segments were lost, which is much more severe than what is seen in a fly *tll* mutant. This result indicates that *Nasonia tll* has a critical and broad patterning function in the posterior and that it may be required for the proper expression of multiple posterior target genes.

In *Drosophila*, *tll* has been shown to be a direct activator of the posterior domain of the gap gene *hunchback* (*hb*) (Margolis et al. 1995). We examined the expression of *Nasonia hb*, which has an expression pattern very similar to that of the fly in the late blastoderm stages (Fig. 3c) (Pultz et al. 2005), in *tll* RNAi embryos. This domain was significantly reduced, but a ventral posterior spot of expression consistently remained. It is possible that this incomplete loss of *hb* expression reflects an incomplete knockdown of *tll*. Alternatively, it could be that, while *tll* is critical for activating *hb* in *Nasonia*, another factor may also contribute. We favor the latter because this pattern of ventral expression of *hb* remaining is not seen in *otd1* RNAi (Lynch et al. 2006), as well as the fact that cuticle phenotypes from the same injection batch showed phenotypes well beyond the range where posterior *hb* acts (abdominal segments 8–10). Since the posterior phenotype of the *hunchback* mutation or *hb* RNAi embryos results, at most, in the loss of the three posterior abdominal segments, *tll* is clearly required for activation of additional targets in the trunk of the embryo.

Another possible target for *tll* in posterior patterning is *caudal*, which, late in the blastoderm stage, is expressed in two stripes at the posterior of the embryo (Fig. 3d) (further description of *Nasonia caudal* expression and function will be published elsewhere). When *tll* was knocked down, the more posterior stripe of expression was reduced or lost, while the anterior stripe was shifted posteriorly (Fig. 3e). In *Drosophila*, there is only one posterior stripe of zygotic *caudal*, which is also lost in *tll* mutant embryos (Mlodzik and Gehring 1987).

These results indicate that some of the targets for posterior *tll* in *Drosophila* have been conserved in *Nasonia*. However, the severity of the *Nasonia tll* pheno-

type indicates that this gene has additional targets that are not affected in *Drosophila*. At this point, it is not clear whether there was ancestrally a larger role for *tll* in posterior patterning. The expanded expression domain of *tll* observed in *Anopheles* seems to be consistent with this idea (Goltsev et al. 2004). In *Tribolium*, the role of *tll* in posterior patterning, if any, is likely to be very different, as it is not expressed at a time consistent with it being involved in regulating posterior patterning genes, and may be required only for proper initiation of the growth zone.

Conclusion

With the advent of widely applicable techniques to examine the expression and function of genes in organisms outside of the traditional model systems, a better understanding of the different mechanisms of embryonic patterning is being gained. In this paper, we have shown that the gene *tailless* has some conserved and mostly divergent features of expression, regulation, and function. Further sampling of a broader palette of insect species, in combination with deeper and more detailed descriptions, should allow for more informed hypotheses regarding the ancestral states of these characteristics, and how they have changed in different lineages.

References

- Bucher G, Scholten J, Klingler M (2002) Parental RNAi in *Tribolium* (Coleoptera). *Curr Biol* 12:R85–R86
- Casali A, 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
- Davis GK, Patel NH (2002) Short, long, and beyond: molecular and embryological approaches to insect segmentation. *Annu Rev Entomol* 47:669–699
- Duffy JB, Perrimon N (1994) The torso pathway in *Drosophila*: lessons on receptor tyrosine kinase signaling and pattern formation. *Dev Biol* 166:380–395
- Goltsev Y, Hsiang W, Lanzaro G, Levine M (2004) Different combinations of gap repressors for common stripes in *Anopheles* and *Drosophila* embryos. *Dev Biol* 275:435–446
- Lynch JA, Brent AE, Leaf DS, Pultz MA, Desplan C (2006) Localized maternal orthodenticle patterns anterior and posterior in the long germ wasp *Nasonia*. *Nature* 439:728–732
- Mahoney PA, Lengyel JA (1987) The zygotic segmentation mutant *tailless* alters the blastoderm fate map of the *Drosophila* embryo. *Dev Biol* 122:464–470
- Margolis JS, Borowsky ML, Steingrimsson E, Shim CW, Lengyel JA, Posakony JW (1995) Posterior stripe expression of *hunchback* is driven from two promoters by a common enhancer element. *Development* 121:3067–3077
- Mlodzik M, Gehring WJ (1987) Expression of the *caudal* gene in the germ line of *Drosophila*: formation of an RNA and protein gradient during early embryogenesis. *Cell* 48:465–478
- Nusslein-Volhard C (1991) Determination of the embryonic axes of *Drosophila*. *Dev Suppl* 1:1–10
- Pignoni F, Baldarelli RM, Steingrimsson E, Diaz RJ, Patapoutian A, Merriam JR, Lengyel JA (1990) The *Drosophila* gene *tailless* is expressed at the embryonic termini and is a member of the steroid receptor superfamily. *Cell* 62:151–163

- Pignoni F, Steingrimsson E, Lengyel JA (1992) Bicoid and the terminal system activate tailless expression in the early *Drosophila* embryo. *Development* 115:239–251
- Pultz MA, Westendorf L, Gale SD, Hawkins K, Lynch J, Pitt JN, Reeves NL, Yao JC, Small S, Desplan C, Leaf DS (2005) A major role for zygotic hunchback in patterning the *Nasonia* embryo. *Development* 132:3705–3715
- Savant-Bhonsale S, Montell DJ (1993) Torso-like encodes the localized determinant of *Drosophila* terminal pattern formation. *Genes Dev* 7:2548–2555
- Schoppmeier M, Schroder R (2005) Maternal torso signaling controls body axis elongation in a short germ insect. *Curr Biol* 15:2131–2136
- Schroder R, Eckert C, Wolff C, Tautz D (2000) Conserved and divergent aspects of terminal patterning in the beetle *Tribolium castaneum*. *Proc Natl Acad Sci USA* 97:6591–6596
- Stevens LM, Frohnhof HG, Klingler M, Nusslein-Volhard C (1990) Localized requirement for torso-like expression in follicle cells for development of terminal Anlagen of the *Drosophila* embryo. *Nature* 346:660–663
- Weigel D, Jurgens G, Klingler M, Jackle H (1990) Two gap genes mediate maternal terminal pattern information in *Drosophila*. *Science* 248:495–498
- Yu RT, McKeown M, Evans RM, Umesono K (1994) Relationship between *Drosophila* gap gene tailless and a vertebrate nuclear receptor Tlx. *Nature* 370:375–379