Dynamic gene expression is required for anterior regionalization in a spider

Matthias Pechmann[1], Alistair P. McGregor[2], Evelyn E. Schwager, Natália M. Feitosa[3], and Wim G. M. Damen[4]

Department of Evolutionary Genetics, Institute for Genetics, University of Cologne, Zülpicher Strasse 47, 50674 Köln, Germany

Edited by Sean B. Carroll, University of Wisconsin, Madison, WI, and approved December 12, 2008 (received for review November 4, 2008)

Patterning of a multicellular embryo requires precise spatiotemporal control of gene expression during development. The gradient of the morphogen bicoid regulates anterior regionalization in the syncytial blastoderm of *Drosophila*. However many arthropod embryos develop from a cellular blastoderm that does not allow the formation of transcription factor gradients. Here we show that correct anterior development of the cellularized embryo of the spider *Achaearanea tepidariorum* requires an anterior-to-posterior wave of dynamic gene expression for positioning the stripes of *hairy*, *hedgehog*, and *orthodenticle* expression. Surprisingly, this dynamic repositioning of the expression of these segmentation genes is blocked in *orthodenticle*^{pRNAi} embryos and no anterior structures are specified in those embryos. Our data suggest that dynamic gene expression across a field of cells is required for anterior regionalization in spiders and provides an explanation for the problem of how positional values for anterior segmentation genes are specified via a morphogen-independent mechanism across a field of cells.

Achaearanea tepidariorum | evolution and development | *hairy* | *orthodenticle* | segmentation

The establishment of positional information to regulate spatiotemporal gene expression patterns is key to metazoan development. The well-characterized segmentation gene cascade realizes this in the *Drosophila* embryo (1). A key feature of the *Drosophila* segmentation gene cascade is the successive refinement of gene expression patterns that ultimately defines the segments along the anterior–posterior body axis. At the top level of the cascade, the transcription factor bicoid (Bcd) forms an anterior-to-posterior gradient that is required for patterning of the anterior of *Drosophila* embryos (2–5). The syncytial blastoderm of the *Drosophila* embryo allows long-range transcription factor gradients, like the Bcd gradient, to directly determine positional values for the expression of target genes that determine where segmental boundaries eventually will arise (1, 6).

This gradient of the morphogen bicoid in *Drosophila* has long been a model for anterior regionalization in arthropods. However, there are 2 problems. First, *bicoid* is unique to higher dipterans and is not found in other insects or arthropods. Recently a solution has been proposed that an anterior-to-posterior orthodenticle (Otd) gradient may play a Bcd-like role in anterior segmentation in other holometabolous insects (7, 8). RNAi knockdown of *otd* in *Nasonia vitripennis* (a wasp) and *Tribolium castaneum* (a beetle) resulted in loss of anterior segments, presumably as a consequence of a shift of target gene expression toward the anterior pole (8). The fast evolving gene *bcd* therefore may have usurped a possible ancestral patterning system involving *otd* in the lineage leading to higher dipterans (7–9).

The second problem is that many arthropods pattern their anterior segments in a cellular environment (10, 11). The gradients of Bcd or Otd acting in holometabolous insects depend on the lack of cell membranes in the syncytial blastoderm embryo that allows diffusion of these transcription factors to form gradients (2–4, 7–9). The blastoderm stages of many other arthropod embryos, however, are cellularized rather than syncytial, which does not allow the formation of transcription factor diffusion gradients (12–16). Very little is known about the mechanisms that account for regionalization of gene expression patterns during anterior patterning in these cellularized embryos.

Here we show that anterior regionalization in the spider *Achaearanea tepidariorum* requires a wave of dynamic gene expression of the segmentation genes *hairy* (*h*), *hedgehog* (*hh*), and *otd*. These genes are initially expressed at the anterior rim of the embryo, but subsequently a single wave of each moves away from the anterior rim toward the posterior. The dynamic gene expression depends on *otd* and is required for anterior regionalization because in *otd* RNAi embryos the expression of these genes does not move posteriorly, but instead persists at the anterior and no anterior structures are specified. Our data suggest that dynamic gene expression across a field of cells is required for anterior regionalization in spiders and thus transcription factor morphogen gradients in insects may be a newly acquired mechanism associated with the switch to syncytial blastoderm development.

Results

Dynamic Anterior Expression of *hairy*, *hedgehog*, and *orthodenticle* in the Spider. Embryos of the spider *Achaearanea* cellularize at the 16-cell stage, before a blastoderm forms (12, 16). To understand anterior regionalization in the cellularized spider embryo we first analyzed the expression of the segmentation genes *hairy* (*h*), *hedgehog* (*hh*), and *orthodenticle* (*otd*). The early anterior expression of these genes exhibits remarkable dynamics.

Achaearanea tepidariorum has 2 *otd* genes, but only *At-otd-1* is expressed during early development. *At-otd-1* expression is first visible in a ring around the germ disk at stage 5 (Fig. 1*A*) (17). This is the anterior border of the embryo (16, 17). At stage 6, *At-otd-1* remains expressed at the anterior rim as the radial symmetry of the germ disk is broken and the embryo opens at its dorsal side (12, 16, 17) (Fig. 1*B*). During stage 7 the germ disk transforms into a germ band and *At-otd-1* expression moves from the anterior rim toward a more posterior position (Fig. 1*C*). The *At-otd-1* stripe eventually is found in a head domain that

Author contributions: M.P. and W.G.M.D. designed research; M.P., A.P.M., E.E.S., and N.M.F. performed research; M.P., A.P.M., and W.G.M.D. analyzed data; and W.G.M.D. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. FM945393 (*At-six3*), FM945394 (*At-Pax6*), FM945395 (*At-lab*), FM945396 (*At-Dfd*), and FM945397 (*At-dac*)].

[1]Present address: Georg-August-Universität Göttingen, Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie GZMB Abteilung für Entwicklungsbiologie, Justus-von-Liebig-Weg 11, D-37077, Göttingen, Germany.

[2]Present address: Institut für Populationsgenetik, Veterinärmedizinische Universität Wien, Josef Baumann Gasse 1, A-1210 Wien, Austria.

[3]Present address: Institut für Entwicklungsbiologie, Universität zu Köln, Gyrhofstrasse 17, D-50923 Köln, Germany.

[4]To whom correspondence should be addressed. E-mail: damen@uni-koeln.de.

This article contains supporting information online at www.pnas.org/cgi/content/full/0811150106/DCSupplemental.

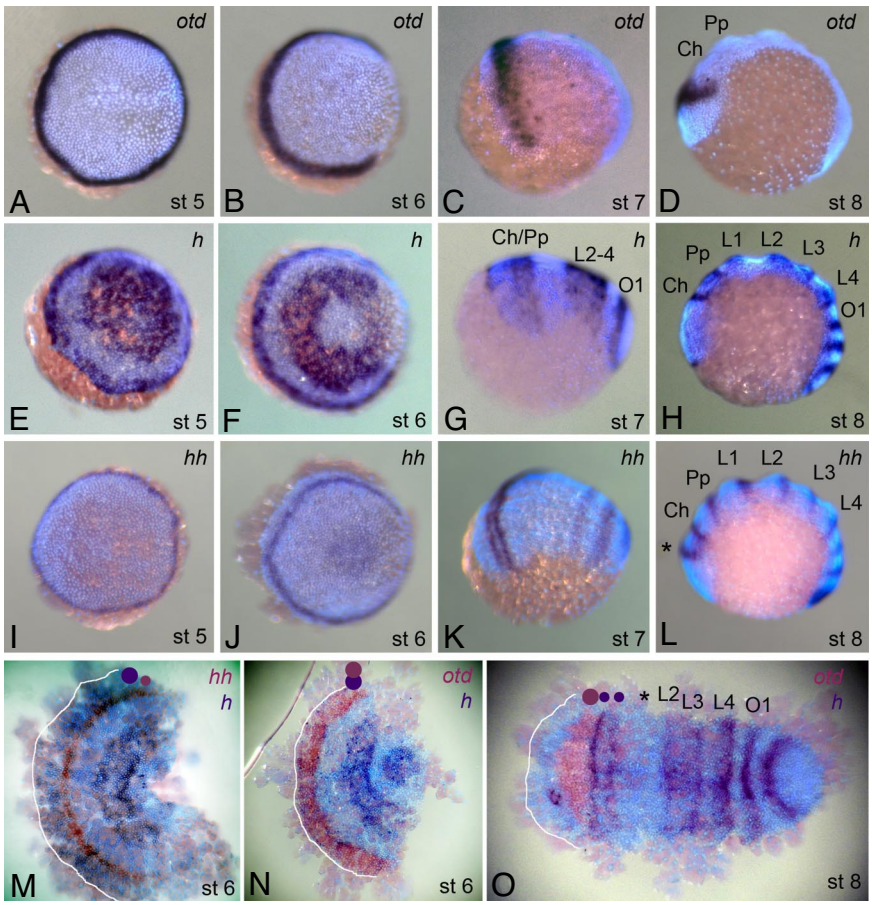

Fig. 1. Dynamic expression of *orthodenticle*, *hairy*, and *hedgehog* during anterior pattering in the spider *Achaearanea tepidariorum*. (*A–D*) *At-otd-1* is expressed in a ring at the rim of the germ disk at stage 5 (*A*); this is the future anterior of the embryo. *At-otd-1* expression remains at the rim when the embryo opens and the radial symmetry breaks (*B*). At stage 7 *At-otd-1* is no longer at the anterior rim of the embryo but is more posterior (*C*) and eventually is in a head domain anterior to the cheliceres at stage 8 (*D*). *At-h* is expressed in a ring at the rim of the germ disk (future anterior) and in a domain in the center of the germ disk (future posterior) (*E*). At stage 6 the anterior expression remains at the rim of the germ disk, but the *At-h* expression clears from the center leaving a broad stripe that corresponds to the future L2–L4 (*F*). At stage 7 (*G*) the anterior stripe of *At-h* is more posterior and splits into 2 stripes that are in the Ch and Pp (*H*). At the posterior *At-h* appears as described previously for *Cs-h* in the spider *Cupiennius salei* (22). *At-hh* also appears as a ring at the rim of the germ disk (*I*), but in contrast to *At-otd-1* and *At-h* is already at stage 6 in a posterior position (*J*). This stripe splits initially into 2 stripes (*K*) and then into 3 stripes that correspond to Ch, Pp, and a head stripe (marked by an * in *L*). (*M*) Double staining for *At-h* and *At-hh*, showing that the *At-hh* stripe, but not yet the *At-h* stripe, is more posterior at stage 6. Both the *At-otd-1* and *At-h* stripe remain expressed at the anterior rim at stage 6 (*N*), but are in different posterior positions later (*O*). The * in *O* marks the position where the L1 stripe will form. The white lines mark the border between embryo and extraembryonic tissue in the panels *M–O*. Ch, cheliceral segment; Pp, pedipalpal segment; L1–L4, walking leg segment 1–4; O1, opistosomal segment 1.

corresponds to the ocular segment and that is clearly anterior to the first appendage-bearing segment of the cheliceres (Fig. 1*D*). Later there is also expression of *At-otd-1* in the ventral midline (data not shown).

At-h and *At-hh* also show dynamic anterior expression patterns that are similar, but not identical to *At-otd-1*. Both *At-h* and *At-hh* are first coexpressed with *At-otd-1* in a ring at the anterior rim of the germ disk in stage 5 embryos (Fig. 1 *E* and *I*). At stage 6, the *At-hh* stripe moves to a more posterior position (Fig. 1*J*), while both *At-otd-1* and *At-h* remain at the rim (Fig. 1 *B*, *F*, and *N*). Double in situ hybridizations for *At-h* and *At-hh* confirm this (Fig. 1*M*). Later *At-h* and *At-otd-1* expression is also found more posteriorly, but at different specific positions for each gene (Fig. 1 *C*, *G*, *N*, and *O*; Fig. 3 *A* and *C*). The anterior stripes of *At-h* and *At-hh* expression then both split into 2 stripes that eventually end up at the location where the cheliceral and pedipalpal segments respectively form (Fig. 1 *H* and *L*). *At-h*, *At-hh*, and *At-otd-1* thus each display a single wave of expression across a field of cells in the anterior spider embryo with individual dynamics for each gene. This is indirectly shown for *At-hh* expression, which is initially coexpressed with *At-h* and *At-otd-1* at the anterior rim (Fig. 1 *A*, *E*, and *I*), but is subsequently found in more posterior cells than *At-h* and *At-otd-1* expression (Fig. 1 *B*, *F*, *J*, and *M*). In addition, *At-h* and *At-otd-1* are coexpressed at the anterior rim until stage 6 (Fig. 1*N*), but eventually end up at different positions (Fig. 1*O*). Extensive cell divisions or cell movements at the anterior rim that "push" the expressing cells posteriorly are unlikely as a single explanation for the dynamics because the stripes of each gene move independently of each other. Furthermore, BrdU incorporation experiments did not show enhanced cell divisions at the anterior rim (supporting information (*SI*) Fig. S1).

***orthodenticle* pRNAi Embryos Lack all Anterior Structures.** Because *otd* is an early anterior patterning gene in insects (7, 8) and the early *At-otd-1* expression is consistent with a role in anterior patterning, we next tested the function of *At-otd-1* using parental RNAi (18). In embryos from mothers that had been injected with *At-otd-1* dsRNA (*otd*^{pRNAi} embryos) the anterior *At-otd-1* expression is strongly reduced (Fig. S2 *A–D*). These *otd*^{pRNAi}

DEVELOPMENTAL BIOLOGY

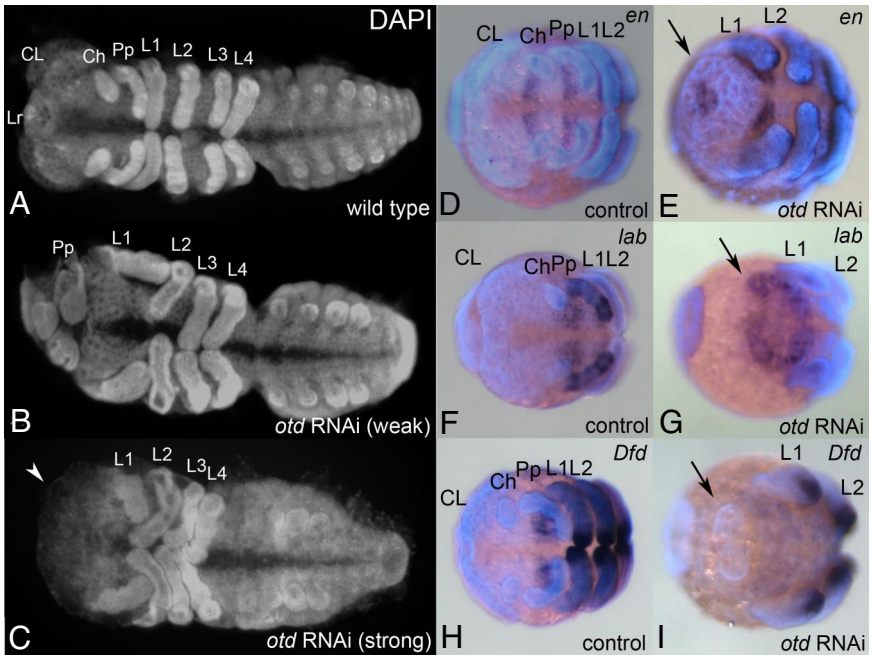

Fig. 2. *At-otd-1* pRNAi results in loss of anterior structures. (*A–C*) Flat-mounted wild-type, weak and strong *At-otd-1*^{pRNAi} embryos, respectively, stained with the nuclear dye DAPI. Structures anterior to the Pp are missing in the *At-otd-1*^{pRNAi} embryos; the Pp and L1 appear to be larger, while more posterior segments are not affected. (*D* and *E*) Wild-type and *At-otd-1*^{pRNAi} embryo stained for the segmental marker *engrailed* (*At-en*). Expression of *At-en* is normal in L1 and more posterior. Anterior to the L1 *At-en* stripes, there is only a single *At-en* domain (arrow in *E*) that presumably represents the Pp expression. (*F*) The Hox gene *labial* (*At-lab*) is a marker for the Pp (*F*); in the *At-otd-1*^{pRNAi} embryo all tissue anterior to L1 expresses *At-lab* (arrow) (*G*). The Hox gene *Deformed* (*At-Dfd*) is expressed in L1–L4 but not in Pp (*H*); in *At-otd-1*^{pRNAi} embryos *At-Dfd* is in the legs L1–L4 but not in the tissue anterior to L1 (arrow) (*I*). The expression of *At-en* (*E*), *At-lab* (*G*), and *At-Dfd* (*I*) strongly suggests that the tissue anterior to L1 is the remnants of Pp. CL, cephalic lobe; Ch, cheliceral segment; Pp, pedipalpal segment; L1–L4, walking leg segment 1–4.

embryos show severe head defects (Fig. 2 *A–E*) and lack structures anterior to the pedipalpal segment, which is the second appendage-bearing segment in spiders (Fig. 2 *C* and *E*). Although in most *otd*^{pRNAi} embryos this segment does not carry an appendage (the pedipalp) or only carries remnants of the appendage, expression of the Hox gene *labial* (*At-lab*) demonstrates that this segment retains pedipalpal identity. *At-lab* is expressed in this segment and extends to the anteriormost border of the *otd*^{pRNAi} embryo (Fig. 2*G*; Fig. S3 *C* and *D*). In wild-type embryos, *At-lab* is strongly expressed in the pedipalpal segment, but not in more anterior structures (19, 20) (Fig. 2*F*; Fig. S3 *A* and *B*). Thus all structures anterior to the pedipalpal segment are missing in *otd*^{pRNAi} embryos. This is confirmed by the lack of *At-Pax6* and *At-six3* expression in these embryos (Fig. S3 *E–L*). *Deformed* (*At-Dfd*) and *dachshund* (*At-dac*) expression shows that the walking leg segments (L1–L4) that are posterior to the pedipalpal segment form normally in *otd*^{pRNAi} embryos (Fig. 2 *H* and *I* and Fig. S3 *M–T*) even if the appendage on L1 is often broader than normal and oddly bent (e.g., Fig. 2*B*).

***The dynamic hairy* and *hedgehog* Expression Depends on *orthodenticle*.** To determine whether *At-otd-1* is required for providing the positional information for *At-h* and *At-hh* gene expression, we investigated *At-h* and *At-hh* expression after *At-otd-1* RNAi. We observed a severe effect on the anterior *At-h* and *At-hh* expression, which is no longer dynamic, but is stationary in *otd*^{pRNAi} embryos. The 2 genes remain expressed at the anterior rim of the germ band and their expression does not move away from the anterior rim (Fig. 3 *B*, *D*, *F*, *H*, and I). Also the *At-otd-1* expression itself does not move toward the posterior in *otd*^{pRNAi} embryos; this is obvious from the very low levels of residual *At-otd-1* transcripts that can still be detected in stage 7 *otd*^{pRNAi} embryos and that do not move from the anterior rim (Fig. S2). Thus, the dynamic repositioning but not the onset of anterior *At-h* and *At-hh* expression depends on *otd*. The posterior *At-h* and *At-hh* stripes are not affected; they are at the same position as in control embryos and show the same dynamics as seen in controls (Fig. 3 *A*, *C*, *E*, and *G*). The dynamics of anterior *At-h*, *At-hh*, and *At-otd-1* expression thus requires *At-otd-1*.

Discussion

Our present data from the spider provide an explanation to the problem of how positional values for anterior segmentation genes are specified via a morphogen-independent mechanism in cellularized arthropod embryos. In *Drosophila* development the syncytial blastoderm allows transcription factor morphogen gradients, like the Bcd gradient, to directly determine positional values for the expression of target genes and where segmental boundaries eventually will arise (1, 6). In other holometabolous insects an Otd gradient may play a role like Bcd in anterior segmentation (7–9). However, the cellular blastoderm of many other arthropods does not allow the formation of diffusion gradients (10, 11) and thus requires a different mechanism for providing positional clues across a field of cells.

We showed that anterior regionalization of the spider requires dynamic spatiotemporal pattern formation that leads to the positioning of the *At-h* and *At-hh* stripes in the anterior spider embryo. The stationary *At-h* and *At-hh* expression at the anterior of the *otd*^{pRNAi} embryos explains the morphological phenotype of these embryos that lack all structures anterior to the pedipalpal segment: In wild-type embryos the posteriormost position of the moving *At-h* and *At-hh* expression defines where the pedipalpal segment forms. However, as a consequence of the inhibition of their dynamic expression, *At-h* and *At-hh* remain at the anterior rim of *otd*^{pRNAi} embryos. Consequently, the pedipalpal segment is incorrectly specified at the extreme anterior,

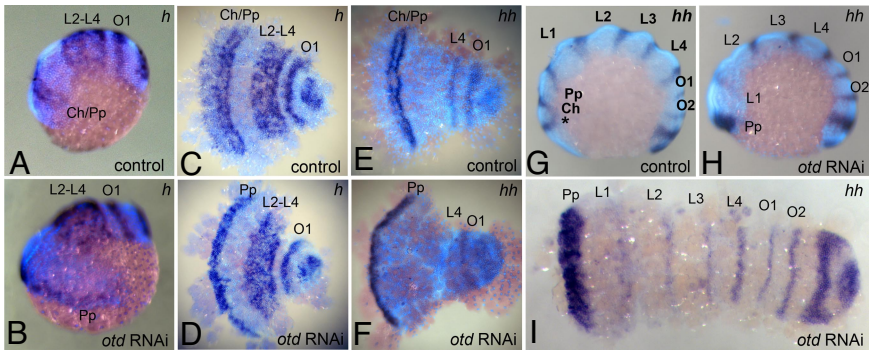

Fig. 3. *At-otd-1* controls the dynamic movement of anterior *At-h* and *At-hh* expression. Expression of *At-h* (*A–D*) and *At-hh* (*E–I*) in control (*A*, *C*, *E*, and *G*) or *At-otd-1*^{pRNAi} (*B*, *D*, *F*, *H*, and *I*) embryos. At stage 7 the *At-h* stripe neither moved from the anterior rim nor split in *At-otd-1*^{pRNAi} embryos (*A* and *B*). The position of the L2–L4 stripe and the opistosomal stripes appear to be unaffected. This is also obvious in the flat preparation in *C* and *D*. Also the *At-hh* stripe did not move to posterior or split in *At-otd-1*^{pRNAi} embryos (*E–I*), while the posterior stripe appears at a normal position. At stage 8, there is still strong *At-hh* expression at the anterior rim, and normal expression in stripes for the segments starting with L1. The * in *G* marks the head domain of *At-hh* expression. CL, cephalic lobe; Ch, cheliceral segment; Pp, pedipalpal segment; L1–L4, walking leg segment 1–4; O1–O2, opistosomal segment 1–2.

and all more normally anterior structures are missing. The entire embryo up to L2 thus consists of only a pedipalpal and an L1 segment (summarized in Fig. 4). Both the pedipalpal and L1 segment seem to be bigger in *otd*^{pRNAi} embryos (e.g., Fig. 2 *A–C*), which is consistent with their specification over a larger field of cells. L2 is the first segment that is completely normal in *otd*^{pRNAi} embryos, which again is consistent with the normal appearance of *At-h*, *At-hh*, and other genes in L2 and more posterior segments. The lack of anterior structures in *otd*^{pRNAi} embryos is not caused by increased cell death, because TUNEL experiments showed no additional apoptosis in *otd*^{pRNAi} embryos (Fig. S4). The missing anterior structures thus are the result of mispatterning caused by the blocking of dynamic gene expression. The dynamic gene expression therefore is required for correct patterning of the field of cells anterior to L2.

Anterior patterning in the spider, like posterior patterning (21–24), thus involves dynamic gene expression. While posterior patterning involves several waves of dynamic gene expression (21–24), anterior pattering involves only a single wave of gene expression for each gene. We showed that *At-otd-1* is required for controlling these dynamics during anterior patterning but it is unlikely that the transcription factor Otd is the only component controlling these dynamics. It is possible that a signaling pathway is also involved, like the Notch signaling pathway in posterior segmentation (21), but it is unclear which signaling pathway because Notch-signaling does not influence anterior segments (25). In the mouse, the *otd* homolog *Otx2* is also a key factor in the head developmental process (26, 27) and is required for proper anterior positioning of the expression of *Dickkopf1* (*Dkk1*), which codes for a secreted protein that acts as a Wnt inhibitor (28, 29). Wnt signaling thus may be a candidate for the anterior patterning in the spider. Furthermore, a gene regulatory network that includes *otx* and *Wnt8* controls a dynamic pattern of gene expression in the specification of endomesodermal territories in sea urchin (30, 31). The dynamic gene expression in the sea urchin is fully explained by positive and negative feedback loops that control wave front generation and decay via transcriptional *cis*-regulatory logic. None of the components involved here meets the definition of a morphogen and the model does not require a graded morphogen concentration distribution (30, 31). Although the precise underlying mechanism for the anterior wave of dynamic gene expression in the spider is not yet solved, similar machinery might be involved. Further research has to dissect the precise mechanisms by which *otd* controls the dynamic expression patterns in the spider.

Interestingly, anterior patterning in the spider is reminiscent of patterning of the eye-antennal imaginal disk in *Drosophila*. The morphogenetic furrow progresses anteriorly in the eye disk, and photoreceptors differentiate in the tissue posterior to the morphogenetic furrow (32, 33). The movement of the morpho-

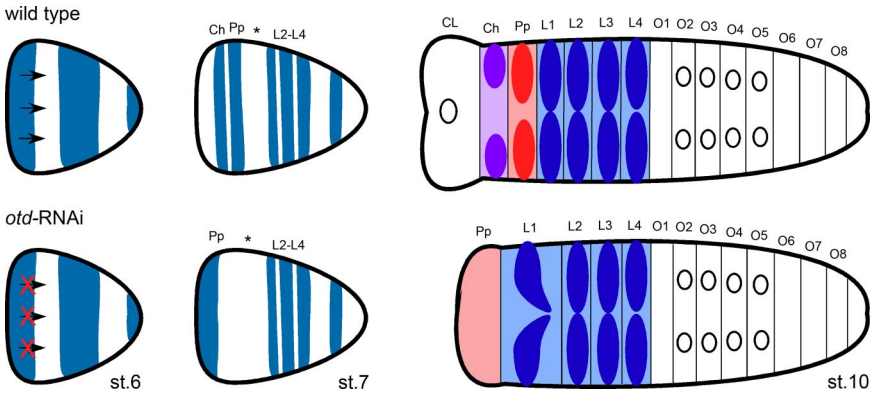

Fig. 4. Schematic drawing of the model for anterior *otd*-dependent patterning in the spider *A. tepidariorum*. A single movement of the anterior expression domains of *hairy* (used as example in drawings), *hedgehog*, and *orthodenticle* is required for proper patterning of the head. This movement depends on *orthodenticle* and after RNAi-mediated knockdown of *otd* the stripes do not move and stay at the anterior rim of the embryo. As a consequence pedipalpal structures are specified at the anterior rim and all structures anterior to the Pp are missing. The * marks the position where the L1 *h* stripe forms slightly later in development. CL, cephalic lobe; Ch, cheliceral segment; Pp, pedipalpal segment; L1–L4, walking leg segment 1–4; O1–O8, opistosomal segment 1–8.

genetic furrow is associated with gene expression that moves dynamically over the eye disk cells (e.g., *atonal*) (33). Thus patterning of both the fly eye disk and the anterior spider head requires a mechanism that involves a wave of dynamic gene expression across a field of cells.

Both insects and spiders require *otd* for the precise positioning of target gene expression required for the correct development of the anterior of the embryo. Therefore the last common ancestor of spiders and insects probably used *otd* as an ancestral organizer of anterior regionalization. However, there is a major difference. In the spider, a field of cells is patterned through dynamic gene expression in a cellularized environment, while in holometabolous insects, a field of nuclei is patterned in a syncytial environment by an Otd morphogen gradient (or Bcd gradient in higher dipterans) (2–4, 8). The transcription factor morphogen gradient as seen in the insects therefore may be a newly acquired mechanism that is associated with the switch to syncytial patterning in the lineage leading to insects.

Methods

Animals. Animals of the common house spider *Achaearanea tepidariorum* (Chelicerata, Aranida, Theridiidae) are kept in a colony in Cologne (24). Embryos are obtained as described (17).

Gene Cloning. *At-otd-1* and *At-en* have been described before (17). Sequences of *At-h* and *At-hh* were available from GenBank. Fragments of *At-Dfd*, *At-lab*, *At-dac*, *At-Pax6*, and *At-six3* were obtained via RT-PCR with degenerate primers (20, 22, 34) and additional sequence via RACE-PCR. Accession numbers are as follows: AB096074 (*At-otd-1*), AB125743 (*At-h*), AB125742 (*At-hh*), AB125741 (*At-en*), FM945396 (*At-Dfd*), FM945395 (*At-lab*), FM945397 (*At-dac*), FM945394 (*At-Pax6*), and FM945393 (*At-six3*).

In Situ Hybridizations, TUNEL, BrdU Incorporation, and RNAi. Whole mount in situ hybridizations and TUNEL were performed with modifications for spider embryos as described previously (18, 35, 36). Parental RNAi was performed as described previously (18). We used dsRNA against 3 different fragments of *At-otd-1*: A large 1042-bp fragment (nt 1–1042), covering almost the complete sequence, and 2 nonoverlapping fragments of 577 bp (nt 113–689) and 350 bp (nt 693-1042). dsRNA against all 3 fragments gave the same phenotypes. In the cell proliferation experiments BrdU labeling reagent (5-Bromo-2′deoxyuridine Labeling and Detection Kit II, Roche) was diluted 1:50 (200 μmol/L) and injected into the perivitelline space of the embryo. Embryos were incubated for 45–60 min and the incorporated BrdU was detected using an AP conjugated anti-BrdU antibody (37).

ACKNOWLEDGMENTS. This work was partially supported by the Deutsche Forschungsgemeinschaft (DFG) via SFB572 of the University of Cologne and the European Union via Research and Training Network Zoonet (MRTN-CT-2004–005624).

1. St Johnston D, Nüsslein-Volhard C (1992) The origin of pattern and polarity in the Drosophila embryo. *Cell* 68(2):201–219.
2. Driever W, Nüsslein-Volhard C (1988) The bicoid protein determines position in the Drosophila embryo in a concentration-dependent manner. *Cell* 54(1):95–104.
3. Berleth T, *et al.* (1988) The role of localization of bicoid RNA in organizing the anterior pattern of the Drosophila embryo. *EMBO J* 7(6):1749–1756.
4. Frohnhöfer HG, Nüsslein-Volhard C (1986) Organization of anterior pattern in the Drosophila embryo by the maternal gene bicoid. *Nature* 324(6093):120–125.
5. McGregor AP (2005) How to get ahead: the origin, evolution and function of bicoid. *Bioessays* 27(9):904–913.
6. Pankratz M, Jäckle H (1993) Blastoderm segmentation in *Development of Drosophila melanogaster*, eds Bate M, Martinez-Arias A (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), pp 467–516.
7. Schröder R (2003) The genes orthodenticle and hunchback substitute for bicoid in the beetle Tribolium. *Nature* 422(6932):621–625.
8. 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(7077):728–732.
9. McGregor AP (2006) Wasps, beetles and the beginning of the ends. *Bioessays* 28:683–686.
10. Peel AD, Chipman AD, Akam M (2005) Arthropod segmentation: beyond the Drosophila paradigm. *Nat Rev Genet* 6(12):905–916.
11. Tautz D (2004) Segmentation. *Dev Cell* 7:301–312.
12. Suzuki H, Kondo A (1995) Early embryonic development, including germ-disc stage, in the Theridiid spider Achaearanea japonica (Bös. et Str.). *J Morphol* 224:147–157.
13. Dearden P, Donly C, Grbic M (2002) Expression of pair-rule gene homologues in a chelicerate: early patterning of the two-spotted spider mite Tetranychus urticae. *Development* 129(23):5461–5472.
14. Browne WE, Price AL, Gerberding M, Patel NH (2005) Stages of embryonic development in the amphipod crustacean, Parhyale hawaiensis. *Genesis* 42:124–149.
15. Ho K, Dunin-Borkowski O, Akam M (1997) Cellularization in locust embryos occurs before blastoderm formation. *Development* 124(14):2761–2768.
16. McGregor A, *et al.* (2008) Cupiennius salei and Achaearanea tepidariorum: Spider models for investigating evolution and development. *Bioessays* 30(5):487–498.
17. Akiyama-Oda Y, Oda H (2003) Early patterning of the spider embryo: a cluster of mesenchymal cells at the cumulus produces Dpp signals received by germ disc epithelial cells. *Development* 130(9):1735–1747.
18. Akiyama-Oda Y, Oda H (2006) Axis specification in the spider embryo: dpp is required for radial-to-axial symmetry transformation and sog for ventral patterning. *Development* 133(12):2347–2357.
19. Damen WGM, Hausdorf M, Seyfarth EA, Tautz D (1998) A conserved mode of head segmentation in arthropods revealed by the expression pattern of Hox genes in a spider. *Proc Natl Acad Sci USA* 95(18):10665–10670.
20. Schwager EE, Schoppmeier M, Pechmann M, Damen WGM (2007) Duplicated Hox genes in the spider Cupiennius salei. *Front Zool* 4:10.
21. Stollewerk A, Schoppmeier M, Damen WGM (2003) Involvement of Notch and Delta genes in spider segmentation. *Nature* 423(6942):863–865.
22. Damen WGM, Weller M, Tautz D (2000) Expression patterns of hairy, even-skipped, and runt in the spider Cupiennius salei imply that these genes were segmentation genes in a basal arthropod. *Proc Natl Acad Sci USA* 97(9):4515–4519.
23. Damen WGM, Janssen R, Prpic NM (2005) Pair rule gene orthologs in spider segmentation. *Evol Dev* 7(6):618–628.
24. McGregor AP, *et al.* (2008) Wnt8 Is Required for Growth-Zone Establishment and Development of Opisthosomal Segments in a Spider. *Curr Biol* 18(20):1619–1623.
25. Oda H, *et al.* (2007) Progressive activation of Delta-Notch signaling from around the blastopore is required to set up a functional caudal lobe in the spider Achaearanea tepidariorum. *Development* 134(12):2195–2205.
26. Acampora D, *et al.* (1998) Murine *Otx1* and *Drosophila otd* genes share conserved genetic functions required in invertebrate and vertebrate brain development. *Development* 125:1691–1702.
27. Matsuo I, Kuratani S, Kimura C, Takeda N, Aizawa S (1995) Mouse Otx2 functions in the formation and patterning of rostral head. *Genes Dev* 9:2646–2658.
28. Mukhopadhyay M, *et al.* (2001) Dickkopf1 is required for embryonic head induction and limb morphogenesis in the mouse. *Dev Cell* 1(3):423–434.
29. Glinka A, *et al.* (1998) Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* 391:357–362.
30. Smith J, Theodoris C, Davidson EH (2007) A gene regulatory network subcircuit drives a dynamic pattern of gene expression. *Science* 318:794–797.
31. Bolouri H (2008) Embryonic pattern formation without morphogens. *Bioessays* 30(5):412–417.
32. Domínguez M, Casares F (2005) Organ specification-growth control connection: new in-sights from the Drosophila eye-antennal disc. *Dev Dyn* 232(3):673–684.
33. Frankfort BJ, Mardon G (2002) R8 development in the Drosophila eye: a paradigm for neural selection and differentiation. *Development* 129(6):1295–1306.
34. Janssen R, Prpic NM, Damen WGM (2004) Gene expression suggests decoupled dorsal and ventral segmentation in the millipede Glomeris marginata (Myriapoda: Diplopoda). *Dev Biol* 268(1):89–104.
35. Damen WGM (2002) Parasegmental organization of the spider embryo implies that the parasegment is an evolutionary conserved entity in arthropod embryogenesis. *Development* 129(5):1239–1250.
36. Prpic NM, Damen WGM (2005) Cell death during germ band inversion, dorsal closure, and nervous system development in the spider Cupiennius salei. *Dev Dyn* 234(1):222–228.
37. Prpic N-M, Schoppmeier M, Damen WGM (2008) The American wandering spider Cupiennius salei: a model for behavioral, evolutionary, and developmental studies. *Emerging Model Organisms: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY), Vol 1, pp 347–372.