

Expression of dorsal-ventral genes during early development of *Rhynchosciara americana* embryos

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Abstract

The establishment of dorsal-ventral polarity in *Drosophila* is a complex process which involves the action of maternal and zygotically expressed genes. Interspecific differences in the expression pattern of some of these genes have been described in other species. Here we present the expression of dorsal-ventral genes during early embryogenesis in the lower dipteran *Rhynchosciara americana*. The expression of four genes, the ventralizing genes *snail* (*sna*) and *twist* (*twi*) and the dorsalizing genes *decapentaplegic* (*dpp*) and *zerknüllt* (*zen*), was investigated by whole-mount *in situ* hybridization. Sense and antisense mRNA were transcribed *in vitro* using UTP-digoxigenin and hybridized at 55°C with dechorionated fixed embryos. Staining was obtained with anti-digoxigenin alkaline phosphatase-conjugated antibody revealed with NBT-BCIP solution. The results showed that, in general, the spatial-temporal expression of *R. americana* dorsal-ventral genes is similar to that observed in *Drosophila*, where *twi* and *sna* are restricted to the ventral region, while *dpp* and *zen* are expressed in the dorsal side. The differences encountered were subtle and probably represent a particular aspect of dorsal-ventral axis determination in *R. americana*. In this lower dipteran *sna* is expressed slightly later than *twi* and *dpp* expression is expanded over the lateral ectoderm during cellular blastoderm stage. These data suggest that the establishment of dorsal-ventral polarity in *R. americana* embryos follows a program similar to that observed in *Drosophila melanogaster*.

Key words

- *Rhynchosciara americana*
- Lower dipteran
- Axis patterning
- Dorsal-ventral genes
- Early embryogenesis

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Presented at the XI Congresso
Brasileiro de Biologia Celular,
Campinas, SP, Brazil, July 15-18,
2004.

Research supported by CNPq, CAPES
and PRONEX.

Received May 27, 2004
Accepted October 19, 2004

The establishment of dorsal-ventral polarity in *Drosophila* embryos is a complex process which involves the action of maternal and zygotically expressed genes. The first step in the establishment of the dorsal-ventral axis is the migration of the oocyte nuclei to a dorsal anterior position. In this position the nuclei initiate a cascade of inter- and intracellular signaling that culminates

with the localization of dorsal protein exclusively in the ventral nuclei of the embryo (1).

The *dorsal* gene plays a particularly crucial role in the specification of dorsal-ventral positional identities during early development. The gradient of nuclear dorsal protein functions as a morphogen that directly activates or represses the transcription of tissue-specific genes in the pathway (2). Along the

ventral midline, where the concentration of nuclear Dorsal protein is the highest, *twist* (*twi*) and *snail* (*sna*) are activated in the presumptive mesoderm. Dorsally, the activity of Dorsal is so low that it can neither activate *twi* and *sna* nor repress *decapentaplegic* (*dpp*) and *zerknüllt* (*zen*), so that only *dpp* and *zen* are expressed. The dorsalizing genes *dpp* and *zen* are responsible for the differentiation of dorsal ectodermal derivatives, including the amnioserosa and dorsal epidermis (3-6). *twi*, *sna*, *zen*, and *dpp* are direct targets of Dorsal, and their regulation represents the earliest subdivision of the dorsal-ventral axis into discrete regions of zygotic gene expression.

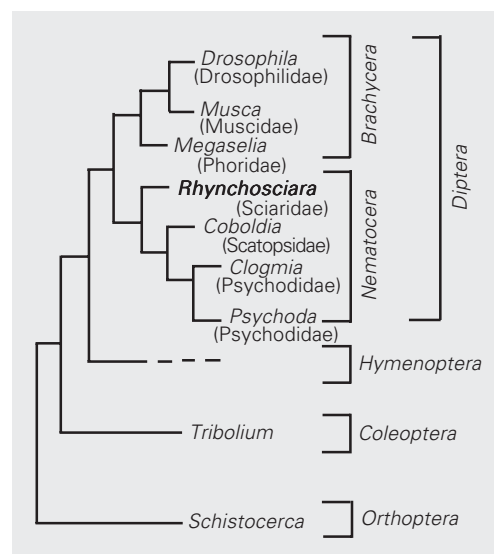
Homologues of maternal dorsal-ventral system genes have been described only in some dipterans while homologues for zygotic genes of this system were also identified in other species of arthropods and some chordates (7). These findings suggest that dorsal-ventral patterning occurs according to a common genetic control in different organisms. However, interspecific differences in the pattern of dorsal-ventral gene expression exist and have been well documented for insects such as *Tribolium* and *Schistocerca gregaria* (8-10).

In the present study, we analyzed the

pattern of expression of some zygotic genes of the dorsal-ventral system in the lower dipteran *R. americana*. In this species the pattern of expression of segmentation genes (gap genes) is quite different from those observed in higher dipterans (Carvalho JC, Rocha DN, Vanario-Alonso CE and Abdelhay E, unpublished results). *R. americana* shows important differences in several stages of embryogenesis when compared to *Drosophila* (11). *R. americana* has a very long development. At 22°C, embryos hatch after 12 days in comparison to the 24 h taken by *Drosophila* embryos and present an unusual developmental pattern, which combines some conserved characteristics similar to early and late *Drosophila* development with a different mid-stage (11). *Rhynchosciara* presents an intermediate step between blastoderm formation and gastrulation. After cellularization the blastoderm starts to differentiate into a short embryonic primordium and an extraembryonic membrane (11). This differentiation culminates in a strong spatial cellular polarization which divides the embryo into a vegetative pole - rich in germplasm with few cells - and a germinative pole that contains the embryo primordium, which is very condensed in this species (11). However, interestingly, the data obtained in the present study suggest that the genetic control of the dorsal-ventral axis seems to be remarkably conserved in *R. americana*, with some minor differences probably due to variations in gene regulation.

To analyze these expression patterns, *R. americana* embryos were staged according to Carvalho et al. (11) and whole-mount *in situ* hybridization was performed as described by Tautz and Pfeifle (12), except that embryo hybridization and washing were performed at 50°C. *Drosophila* cDNA probes for the genes under study were obtained from Dr. Stephen Small (New York University) and were used to transcribe sense and antisense RNA probes. We used sense RNA under the same stringency and temperature

Figure 1. Phylogenetic tree showing the closeness of the species considered in the present study. Adapted from Rohr et al. (14).



conditions as control and no labeling could be detected. The photographic images were digitized and then edited electronically using Adobe Photoshop software.

We examined the expression of *twi*, *sna*, *dpp*, and *zen* in the early embryogenesis of *R. americana*. For whole-mount *in situ* hybridization we used anti-sense mRNA probes obtained from *D. melanogaster* cDNAs. Given the phylogenetic closeness of the two species (Figure 1), the use of heterologous probes suited our purpose and we were able to analyze the expression of all four genes.

sna gene expression was detected during a short period of *R. americana* early embryogenesis. This expression was confined to the syncytial blastoderm stage (8 h) and *sna* transcripts were localized in the ventral region of the embryo (Figure 2A). Expression of *twi* mRNA was also detected in the ventral region of the embryo, although at a later time (12-18 h). *twi* gene expression was detected during blastoderm cellularization of embryo nuclei (Figure 2B). These results contrast with those obtained for *Drosophila*, in which both the *sna* and *twi* genes are initially expressed at the 11th nuclear cleavage (4). In *Drosophila*, the cellular blastoderm forms during the 14th mitotic cycle and thus *sna* and *twi* are expressed early in the syncytial blastoderm. This temporal difference in *twi* expression could be related to the longer embryogenesis of *R. americana*. Probably the temporal regulation of *sna* and *twi* expression in *R. americana* is more evident than in *Drosophila* due to the longer time taken by cellularization (24 h in *R. americana* versus 4 h in *D. melanogaster*).

In *Drosophila*, Dorsal directly activates *sna* and *twi* expression and the width of the *sna* domain depends on *twi*. However, the initiation of *twi* expression is essentially normal in *sna* mutant embryos and the initiation of *sna* expression is normal in *twi* mutant embryos (6). Thus, the temporal difference observed in *R. americana* for *sna* and *twi* expression is a particular aspect of this spe-

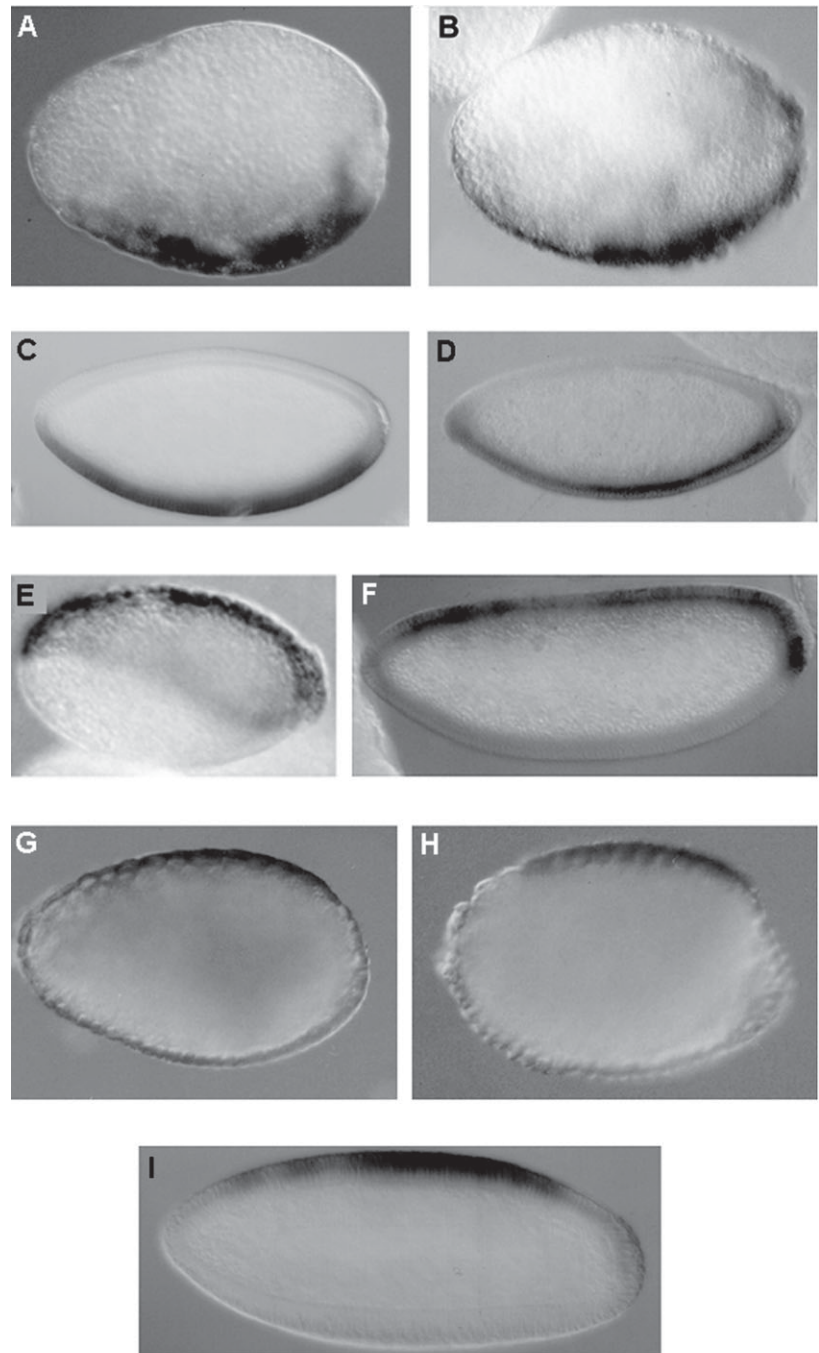


Figure 2. Analysis of dorsal-ventral gene expression in a *Rhynchosciara americana* embryo by whole-mount *in situ* hybridization. Anterior is to the left. A-B, *sna* and *twi* mRNA distribution in the syncytial blastoderm (8 h) and cellularization of nuclear blastoderm (12-18 h) stages, respectively. C-D, *sna* and *twi* expression in a *Drosophila melanogaster* embryo during the cellular blastoderm stage. E-F, *dpp* mRNA distribution in mid-cellular blastoderm stages of *R. americana* and *D. melanogaster* embryos, respectively. G-H, *zen* mRNA distribution during the early cellular blastoderm (24 h) and late cellular blastoderm stage (48 h) of an *R. americana* embryo, respectively. I, *zen* expression in a *D. melanogaster* embryo during the late cellular blastoderm stage.

cies that reflects a modification in the way these genes are regulated. Nevertheless, we can say that the expression of the *sna* and *twi* genes is spatially and temporally conserved because both initiate their expression on the ventral surface and still in the precellular blastoderm stage, as they are in *Drosophila*.

The early expression of the dorsalizing genes *dpp* and *zen* is not detected until cellular blastoderm stage of *R. americana* development. In contrast, in *Drosophila* both genes are initially expressed at the precellular blastoderm stage prior to nuclear cycle 14. However, similar to *Drosophila*, in *R. americana*, the *dpp* and *zen* domain of expression was localized in a dorsal region of the embryonic blastoderm (Figure 2E and G-H). During the cellular blastoderm stage the distribution of *dpp* mRNA and *zen* transcripts extends into the anteroposterior region of the embryo. In early stages *dpp* transcripts always occupy a broader domain of expression than *zen* mRNA. Surprisingly, while in *Drosophila* *dpp* expression is restricted to the dorsal non-neurogenic ectoderm region, in *R. americana* *dpp* expression seems to expand over the lateral ectoderm. Probably this expansion will be later reduced when the cells from the ventral region will migrate to the

dorsal side of the embryo (11).

In *Drosophila*, *dpp* and *zen* are expressed in a region of the same extension. In later stages the *zen* pattern is refined, occupying a more dorsal and restricted area (6). This refinement seems not to occur in *R. americana*, reflecting fine modifications in the control of regulation that has been attained by evolutionary adaptation of the embryogenesis mode of *R. americana*. Previous study by Stauber et al. (13) in non-cyclorraphan flies has suggested that *zen* has other functions than dorsal-ventral patterning. *zen* has been proposed to substitute the *bicoid* morphogen in anteroposterior patterning. This seems not to be the case in *R. americana* since our results did not show any early *zen* expression. Taken together, the present data indicate that in general the early expression patterns of dorsal-ventral genes are conserved in *R. americana*.

Acknowledgments

We thank Terezinha Maria Castro-Silva for technical assistance. We are grateful to Dr. Stephen Small (New York University, USA) for providing the cDNAs.

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