Developmental Biology 381 (2013) 189–202

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Patterning the dorsal–ventral axis of the wasp *Nasonia vitripennis*

Thomas Buchta [a,1], Orhan Özüak [a,1], Dominik Stappert [a], Siegfried Roth [a,*], Jeremy A. Lynch [a,b,**]

[a] Institute for Developmental Biology, University of Cologne, Zülpichstrasse 47b, 50674 Cologne, Germany
[b] Department of Biological Sciences, University of Illinois at Chicago, 900S Ashland Ave., Chicago, IL 60607, USA

ARTICLE INFO

Article history:
Received 29 November 2012
Received in revised form
14 May 2013
Accepted 24 May 2013
Available online 2 June 2013

Keywords:
Nasonia
Tribolium
Fate map
Dorsal–ventral patterning
Mesoderm
Extraembryonic
Patterning
Embryo

ABSTRACT

Regulatory networks composed of interacting genes are responsible for pattern formation and cell type specification in a wide variety of developmental contexts. Evolution must act on these regulatory networks in order to change the proportions, distribution, and characteristics of specified cells. Thus, understanding how these networks operate in homologous systems across multiple levels of phylogenetic divergence is critical for understanding the evolution of developmental systems. Among the most thoroughly characterized regulatory networks is the dorsal–ventral patterning system of the fly *Drosophila melanogaster*. Due to the thorough understanding of this system, it is an ideal starting point for comparative analyses. Here we report an analysis of the DV patterning system of the wasp, *Nasonia vitripennis*. This wasp undergoes a mode of long germ embryogenesis that is superficially nearly identical to that of *Drosophila*, but one that was likely independently derived. We have found that while the expression of genes just prior to the onset of gastrulation is almost identical in *Nasonia* and *Drosophila*, both the upstream network responsible for generating this pattern, and the downstream morphogenetic movements that it sets in motion, are significantly diverged. From this we conclude that many network structures are available to evolution to achieve particular developmental ends.

Introduction

All bilaterally symmetric animals face the problem of setting up two orthogonal body axes during embryogenesis. The mechanisms employed in these processes are variable across evolutionary time, with embryological and environmental factors influencing the strategies employed in various lineages. In order to understand how axis determination and patterning processes can change in the course of evolution, a comparative approach that incorporates highly detailed descriptions of homologous developmental processes is required. The establishment and patterning of the dorsal–ventral axis of the fruit fly *Drosophila melanogaster* is one of the most thoroughly described embryonic patterning systems among the bilateria, and thus serves as a valuable point of comparison for studies focused on the evolution of patterning processes.

The chain of events that leads to cell fate determination along the DV axis of the *Drosophila* (and other insects (Lynch et al., 2010)) embryo begins in the ovary (reviewed in (Roth and Schüpbach, 1994)). Here, *gurken* mRNA localized anteriorly and asymmetrically with regard to the short axis of the oocyte, leads to the localized activation of EGF signaling in the overlying follicle cells, and this signal specifies the future dorsal side of the egg (Neuman-Silberberg and Schüpbach, 1993; Roth, 2003). EGF signaling also precisely restricts the expression of the sulfotransferase *pipe* to the ventral follicle cells, which in turn leads to a localized activation of a protease cascade in the perivitelline space (Sen et al., 1998; Cho et al., 2010). The outcome of the activated protease cascade is the graded cleavage, and thus activation, of the Toll ligand Spätzle (Spz) in the ventral half of the perivitelline space (Moussian and Roth, 2005).

In the early embryo, cleaved Spz protein binds the maternally provided Toll receptor present in the plasma membrane. Upon Toll activation by Spz the IκB homolog Cactus (Cact) becomes phosphorylated and degraded, which in turn leads to the release and translocation of the NF-κB transcription factor Dorsal to the nucleus, creating a stable DV gradient of nuclear Dorsal with peak levels at the ventral midline (Moussian and Roth, 2005). Dorsal acts as a morphogen, directly regulating around 50 genes in a concentration dependent manner (Stathopoulos et al., 2002; Hong et al., 2008).

Dorsal target genes contain enhancers that vary in the number, affinity, and arrangement of Dorsal binding sites and determine their sensitivity to nuclear Dorsal concentrations (Stathopoulos and Levine, 2004; Hong et al., 2008). The expression of genes with

* Corresponding author.
** Corresponding author.
E-mail addresses: siegfried.roth@uni-koeln.de (S. Roth). jlynch42@uic.edu (J.A. Lynch).
[1] These authors contributed equally.

0012-1606/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ydbio.2013.05.026

enhancers containing low affinity Dorsal binding sites can only be activated by high levels of nuclear Dorsal, and are thereby restricted to the ventral region of the embryo. Examples are genes like *twist* and *snail*, which are involved in the specification and morphogenesis of mesoderm. Genes such as *ventral neuroblasts defective (vnd)* and *brinker (brk)* that react to moderate and low nuclear Dorsal concentrations are characterized by enhancers containing high affinity affinity Dorsal binding sites in combination with binding sites for bHLH, Supressor of Hairless and/or ETS domain transcription factors. They have lateral, stripe like expression domains, due to repression by Snail ventrally. Finally, genes like *short gastrulation (sog)* and *zerknuellt (zen)*, that react to low levels of Dorsal, are characterized by enhancers containing high affinity Dorsal binding sites with either activating (e.g. *sog* enhancer) or repressing influence (e.g. *zen* enhancer) depending on the presence of closely linked co-activator or co-repressor binding sites, respectively (Rusch and Levine, 1996; Reeves and Stathopoulos, 2009).

Several Dorsal target genes are transcription factors that interact with each other and which further refine and elaborate the expression of downstream target genes. Dorsal not only patterns the ventral and lateral parts of the *Drosophila* embryo, but also plays a major role in regulating the BMP signaling pathway, which patterns the dorsal half of the embryo. By activating the BMP inhibitor *sog* in a broad lateral domain and repressing the BMP2/4-like ligand *decapentaplegic (dpp)* and the metalloprotease *tolloid (tld)* in the ventral half of the embryo, Dorsal facilitates the establishment of a gradient of BMP activation with a sharp peak at the dorsal midline (O'Connor et al., 2006).

Althoughh the *Drosophila* DV patterning system is one of the best understood gene regulatory networks (GRNs), and thus is the gold standard to which other insects will be compared, the fly is not typical of insects in many respects. It shows highly derived features and undergoes a long germ mode of embryogenesis, which is only found among holometabolous insects. In this type of embryogenesis all segments are specified simultaneously within the blastoderm stage embryo (Davis and Patel, 2002). In contrast, all hemimetabolous and some holometabolous insects, such as the beetle *Tribolium castaneum*, undergo a short germ mode of embryogenesis. In this mode of embryogenesis only the head and thoracic segments are specified prior to gastrulation, while the remaining segments are generated and patterned progressively after gastrulation (Davis and Patel, 2002). Thus, short germ patterning requires at least two steps in DV patterning: one acting at the blastoderm stage which partitions the head and thoracic segments, and a second one being active in the post-gastrulation growth zone.

The Dorsal protein of *Tribolium* (Tc-Dorsal), like its *Drosophila* counterpart, forms a gradient during early embryogenesis, and is involved in patterning cell fates along the DV axis of the early embryo (Chen et al., 2000; Nunes da Fonseca et al., 2008). However, the function of Tc-Dorsal differs from that of fly Dorsal in two fundamental ways. First, the Tc-Dorsal gradient is dynamic over developmental time, while the shape of the *Drosophila* gradient is relatively stable (Chen et al., 2000; Kanodia et al., 2009; Liberman et al., 2009). The domain of nuclear Tc-Dorsal is initially weak and shallowly graded, then progressively shrinks to form a steeply graded stripe straddling the ventral midline, before finally disappearing completely just prior to gastrulation. The dynamics of the Tc-Dorsal gradient are a result of a feedback loop of zygotic target genes of Tc-Dorsal, which include both its upstream activating receptor Tc-Toll, and its inhibitor, Tc-Cactus (Nunes da Fonseca et al., 2008). *Tc-Toll*, *Tc-cactus*, and at least one additional zygotic target of Tc-Dorsal (*Tc-twist*) are expressed in dynamic patterns that seem to follow the changes in the Tc-Dorsal gradient (Nunes da Fonseca et al., 2008).

A second difference between the fly and beetle systems is that Tc-Dorsal is only directly involved in specifying cell fates along the DV axis in a fraction of the embryo, since the Tc-Dorsal nuclear gradient is only present prior to gastrulation, and does not operate in the growth zone (Chen et al., 2000). In contrast, fly Dorsal assigns DV cell fates to all segmental primordia prior to gastrulation.

These differences between *Tribolium* and *Drosophila* Dorsal function lead to questions about which characteristics of the *Drosophila* DV patterning system are due to its mode of embryogenesis, and which characteristics of the *Tribolium* system are truly representative of the ancestral mode for insects.

To begin to address these questions, we have initiated an examination of the DV patterning process of the wasp *Nasonia vitripennis*. This wasp has a mode of long germ embryogenesis similar to, but independently derived from, that of the fly, which makes it an ideal model for understanding the patterning requirements for long germ embryogenesis (Lynch et al., 2012). On the other hand, it is a member of the Hymenoptera, the most basally branching order of the Holometabola (fully metamorphosing insects), and *Nasonia* thus represents a key phylogenetic sampling point for reconstructing features of the ancestral DV patterning mechanism within this clade (Lynch et al., 2012).

To fully understand how establishment and patterning of the DV axis come about at a functional level, the process must first be well described observationally. Only after such a thorough description can perturbations of the system be robustly interpreted. Thus, to provide a basis for future functional experiments, and to gain insights into how DV axial patterning comes about in *Nasonia*, we have cloned and analyzed the expression of *Nasonia* orthologs of *Drosophila* DV marker genes covering the entire embryonic axis. We have found that the expression patterns of these genes just prior to gastrulation are highly similar between *Nasonia* and *Drosophila*. However, our results also show that some aspects of the gene regulatory networks both upstream and downstream of this conserved arrangement have diverged significantly between the wasp and the fly. Finally, incorporation of gene expression data from the beetle *Tribolium* into this comparative work has shed light onto features and components of DV patterning that were likely present in the last common ancestor of the Holometabola.

Results

Characterization of early Nasonia embryogenesis

In order to best be able to interpret the dynamics of gene expression, the process of *Nasonia* embryogenesis needed further characterization. We took two approaches to this end. One was timed egg collections and DAPI staining to characterize the stages present at different time points at 29 °C (the temperature at which embryos were laid and incubated) (Fig. S1). The other was to take advantage of the optically clear embryo of *Nasonia* to make time lapse DIC movies of embryogenesis (supplemental movie 1). These two approaches were complementary, and led us to the same conclusions about early *Nasonia* embryogenesis, that were generally consistent with previous work (Bull, 1982). Like *Drosophila*, there are rapid, synchronous syncytial divisions of nuclei before the onset of gastrulation. In *Nasonia*, there are 12 of these divisions, rather than 13 observed in *Drosophila*. The initial pole cell bud occurs after 6 divisions (02:00, supplemental movie 1), one cycle prior to the appearance of the rest of the nuclei on the egg surface (02:20, supplemental movie 1). This is in contrast to *Drosophila*, where the pole cells form simultaneously with the arrival of nuclei to the embryo surface.