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Gaining New Insights into Primitive Strategies for Embryonic Axis Specification Using the Wasp *Nasonia*

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ABSTRACT

The evolution of genetic networks is a fascinating and complex topic that has long intrigued researchers. The genetic network controlling early embryonic patterning in *Drosophila* represents one of the best understood networks in developmental biology. Thus, the realization that major components of the network are not conserved features of insect embryogenesis provided the scientific field with an incredible opportunity to begin comparative studies between the well-studied *Drosophila* network and the genetic networks of other insect species. Moreover, the tremendous diversity among insects provides a wide variety of species to sample the conserved and novel developmental features that have evolved over time. The application of genetic screens, transgenic analysis and in particular, the development of pRNAi in various insect model systems has also contributed significantly to the advancement of the field of evolution and development. The results presented in recent reports regarding *Nasonia*, *Tribolium*, *Oncopeltus* and *Gryllus* embryonic patterning have shown the power of comparative studies between different insects for studying evolution and development. This review will focus on the establishment of the wasp *Nasonia vitripennis* as a powerful model system for elucidating the various biological strategies employed during insect embryogenesis. Moreover, work presented throughout this review will highlight important results regarding comparative studies between the fruit fly and the wasp.

Keywords: *bicoid*, *caudal*, *giant*, *nanos*, *orthodenticle*, segmentation

CONTENTS

INTRODUCTION

One of the first steps in the development of a single-celled embryo is the establishment of the anterior-posterior (AP) and dorsal-ventral (DV) axes. Subsequently, a complex genetic program patterns these axes to ultimately give rise to a basic adult body plan. The mechanisms employed by the embryo to achieve this initial polarization have long fascinated developmental biologists and various strategies have now been identified among different animal species for achieving this general polarization of the embryo. Moreover, it is clear that while some of these mechanisms are conserved throughout the animal kingdom, other patterning functions have significantly evolved.

Insects provide a fascinating group of organisms in which to study the diversity, as well as the conservation of mechanisms employed for both the polarization and early patterning of the embryo. The early patterning of the fruit fly *Drosophila melanogaster* is one of the best described developmental systems, providing an excellent comparison

Received: 31 January, 2007. Accepted: 14 February, 2007.

Invited Review

for work on the evolution of the patterning networks among insects (Davis and Patel 2002; Lynch and Desplan 2003a). It has become clear that the crux of the *Drosophila* patterning network, the gene *bicoid*, as well as the basic mode of development within the fruit fly are not well conserved developmental features among insects (Stauber *et al*. 2002; Lynch and Desplan 2003b). Many researchers have thus sought to identify a more general ancestral patterning network and mode of development within insects (Liu and Kaufman 2005b). To this end, a wealth of information has been reported regarding the development of the beetle *Tribolium*, the cricket *Gryllus*, as well as the milkweed bug *Oncolpeltus*. In addition to descriptive reports regarding the temporal and spatial expression patterns of various genes during embryogenesis, elegant studies have made use of genetic mutant screens and RNA interference to uncover the functions of developmental genes and how their roles within their respective patterning network compare to their counterparts in the *Drosophila* network (Sulston and Anderson 1996; Schulz *et al*. 1998; Wolff *et al*. 1998; Schroder *et al*. 2000; Schroder 2003; Liu and Kaufman 2004; Cerny *et al*. 2005; Liu and Kaufman 2005a, 2005b; Mito *et al*. 2005; Shinmyo *et al*. 2005; van der Zee *et al*. 2005; Mito *et al*. 2006; Choe and Brown 2007).

Among the various insects recently adapted as model organisms for use in comparative studies of embryogenesis with *Drosophila*, the wasp *Nasonia vitripennis* has been successfully used to identify mechanisms utilized for the initial polarization of the insect embryo and to identify features of the early patterning network. Studies presented throughout this review will highlight conserved aspects of insect embryogenesis including the use of gradients, cross repression between zygotic genes to refine expression boundaries, as well as the reliance on localization of maternal mRNAs. Additionally, unique characteristics of *Nasonia* embryogenesis have been uncovered which point to the plasticity of the genetic network responsible for insect embryogenesis throughout evolution (Pultz *et al*. 2005; Lynch *et al*. 2006a, 2006b; Olesnicky *et al*. 2006). In order to fully understand the impact of the *Nasonia* system and the recent data that have emerged regarding the development of the wasp in contrast to the embryonic network of *Drosophila*, it is first necessary to describe the well-known details of *Drosophila* embryogenesis.

DIFFERENT MODES OF EMBRYOGENESIS: SHORT VS. LONG GERM

A great deal of diversity is seen in mechanisms of embryogenesis among insects, ranging from polyembryonic embryogenesis, where thousands of embryos are derived from a single egg, to the highly derived system of long germ embryogenesis, characteristic of *Drosophila*. Despite this variety in insect embryogenesis, most insects can be categorized into 2 different developmental groups: short/intermediate germ and long germ insects (Grbic 2003; Liu and Kaufman 2005b). Short germ and intermediate germ insects are represented throughout basal insect orders. This is in contrast to long germ insects, which are confined to some holometabolous insect species. This suggests that short or intermediate germ embryogenesis is the ancestral mode of development (Liu and Kaufman 2005b) while long is a derived state.

In short germ embryogenesis, the majority of the embryo is occupied by extraembryonic membranes, while the embryo develops in the posterior of the egg and only anterior embryonic structures are patterned in the early syncytial environment of the germ anlage. Later, after gastrulation, abdominal and posterior structures are formed in a cellularized environment through a region in the posterior of the germ rudiment termed the "growth zone". The growth zone undergoes extensive elongation during which abdominal segments become specified in an anterior to posterior progression. Within short and intermediate germ insects, there is a difference in the number of segments patterned syncytially, prior to gastrulation. Short germ embryos pattern typically only head segments prior to gastrulation, while intermediate germ insects will also pattern thoracic segments within the syncytial germ anlage (Davis and Patel 2002). Additionally, the proportion of the egg devoted to either the embryonic anlage or the extraembryonic membranes, varies among short and intermediate germ insects (Davis and Patel 2002; Liu and Kaufman 2005b).

Short germ embryogenesis is in sharp contrast to the derived long germ mode of patterning found in both *Drosophila* and *Nasonia*, where the embryo occupies the entire egg and the entire adult fate map is specified completely within a syncytial environment prior to gastrulation. As all segments are patterned in the syncytial blastoderm embryo, the long germ embryo lacks a posterior growth zone. The honeybee was the first organism in which long germ embryogenesis was described. In the honeybee, the entire length of the egg is occupied by the embryo and it undergoes almost no germ band elongation during gastrulation (Davis and Patel 2002). This is in contrast to *Drosophila*, the most studied long germ embryo, which does undergo germ band elongation, during which the embryo is lengthened though a dramatic series of cellular rearrangements. At the conclusion of germ band elongation, the embryo returns to its original size as it continues to pattern the insect body plan. Interestingly, however, unlike *Drosophila*, where all segments are patterned simultaneously, the honey bee undergoes development in an anterior-posterior progression, which is reminiscent of short germ embryogenesis, underlining an obvious diversity among insects within the mechanism of long germ embryogenesis (Davis and Patel 2002).

The diversity among these groups of insects that undergo drastically different modes of embryogenesis highlights the need for sampling a broader range of species within both the short and long germ categories to obtain a greater understanding and dissection of the genetic networks involved in embryogenesis. Such information will be important for identifying conserved features among insect embryogenesis and for the general understanding of the evolution of genetic networks.

THE PATTERNING NETWORK OF *DROSOPHILA MELANOGASTER*

Patterning along the AP axis in the *Drosophila* embryo involves the coordinate inputs of an intricate genetic hierarchy. The initial coordinating information in this patterning cascade is communicated by the maternal system, i.e. genes whose function is required in the mother, during oogenesis, for the proper patterning of the embryo. Opposing gradients of maternal signaling molecules are loaded into the oocyte during oogenesis. Upon fertilization, an anterior to posterior gradient of the homeodomain transcription factor Bicoid (Bcd) is created (Driever and Nusslein-Volhard 1988a). An opposing posterior to anterior gradient is formed when Bcd represses translation at the anterior of the embryo of the homogeneously loaded maternal *caudal* (*cad*) mRNA, which encodes a homeodomain transcription factor (Macdonald and Struhl 1986; Mlodzik and Gehring 1987). Similarly, maternal *hunchback* (*hb)* mRNA is homogenously present in the early embryo. However, the translation of this zinc finger transcription factor is prevented in the posterior half of the embryo by *nanos* (*nos*), a factor required for abdomen formation (Irish *et al*. 1989; Lehmann and Nusslein-Volhard 1991; Rivera-Pomar and Jackle 1996).

The restricted expression of these maternal factors serves to divide the embryo in domains, with Bcd and Hb positioned at the anterior and Cad and Nos occupying the posterior half of the embryo. The information encoded by these factors is then interpreted by downstream targets, termed gap genes. These are the first zygotically expressed segmentation genes that serve to subdivide the embryo into large subdomains. In the anterior, gap genes such as the homeodomain transcription factor Orthodenticle (Otd) become expressed in response to the Bcd gradient (Driever *et*

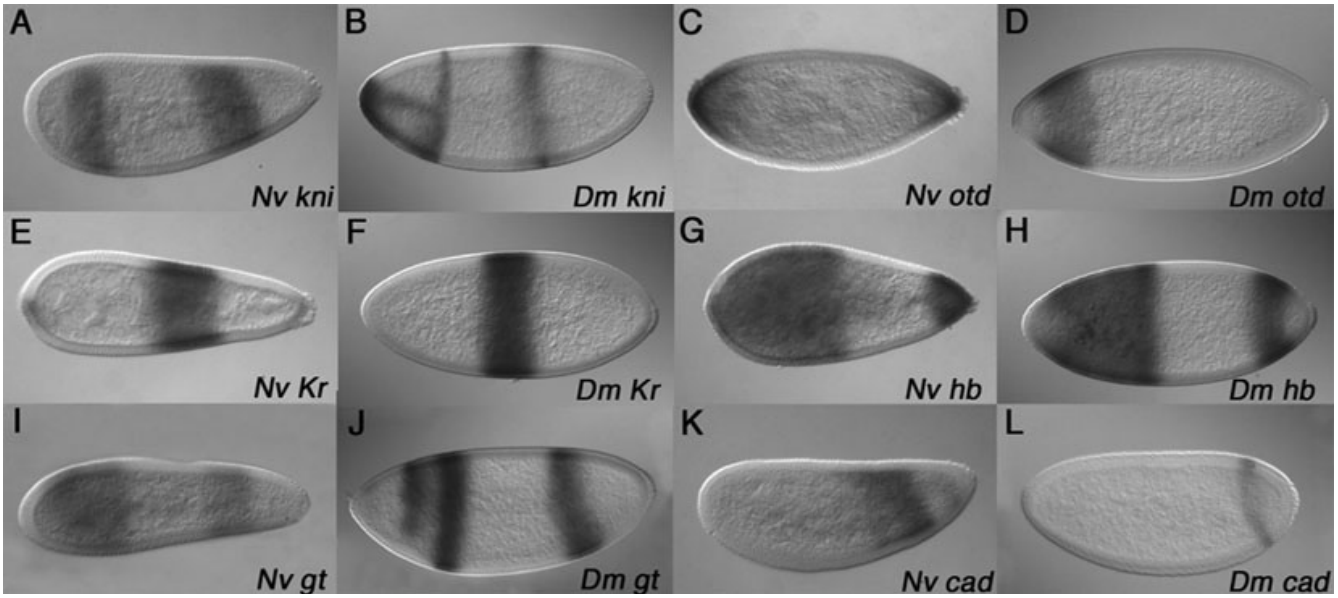

Fig. 1 A comparison of *Nasonia* and *Drosophila* zygotic gap gene expression patterns. Wild type expression patterns of *Nasonia kni* (**A**), *Drosophila kni* (**B**), *Nasonia otd* (**C**), *Drosophila otd* (**D**), *Nasonia Kr* (**E**), *Drosophila Kr* (**F**), *Nasonia hb* (**G**), *Drosophila hb* (**H**), *Nasonia gt* (**I**), *Drosophila gt* (**J**), *Nasonia cad* (**K**), and *Drosophila cad* (**L**).

al. 1989; Driever and Nusslein-Volhard 1989; Cohen and Jurgens 1990). Thoracic and central gap genes such as *Krüppel* (*Kr*) are activated in response to the coordinate functions of Hb and Bcd (Hoch *et al*. 1991). In the posterior abdomen, the embryo is divided into the expression domains of *knirps* (*kni*) and *giant* (*gt*). Activation of the *kni* posterior expression domain is achieved by the functions of both Bcd and Cad (Rivera-Pomar *et al*. 1995; Schulz and Tautz 1995; Schroeder *et al*. 2004).

The domains of gap gene expression become refined as the gap genes regulate one another to form more precise expression boundaries (**Fig. 1**) (Treisman and Desplan 1989; Hulskamp *et al*. 1990; Hulskamp and Tautz 1991; Kraut and Levine 1991a). This precise subdivision of the embryo initiates the next tier of the patterning hierarchy by refining spatial information. Pair rule gene expression is activated in response to the gap genes and functions to subdivide the embryo into even smaller segmental domains. Pair rule genes, such as *even skipped* (*eve*) and *fushi tarazu* (*ftz*) are expressed in a seven stripe pattern along the antero-posterior axis of the embryo (Carroll and Vavra 1989; Carroll 1990; Hader *et al*. 1998). Once again, cross regulation between the pair rule genes serves to create sharp expression boundaries in the form of stripes along the antero-posterior axis. This next tier of patterning genes will continue to refine the pattern by activating its downstream targets, the segment polarity genes. Segment polarity genes such as *engrailed* or *wingless* will finally provide positional information to every cell along this axis (Hidalgo and Ingham 1990; Schuske *et al*. 1994; Rivera-Pomar and Jackle 1996; Davis and Patel 2002; Choe and Brown 2007).

THE BCD MORPHOGEN GRADIENT

Morphogenetic gradients represent a powerful strategy employed by cells to supply both positional and instructional information to surrounding cells or tissues. The use of this mechanism has been uncovered as a common theme in developmental programs ranging from early embryogenesis to neurogenesis. The establishment of a graded signal allows for cells to interpret their location relative to the signal source. Moreover, a signaling gradient, in addition to supplying positional information, instructs a variety of cell fate decisions that depend on the concentration of signal present (Ashe and Briscoe 2006).

One well-studied example of a morphogen is the Bicoid gradient, which is established in the early *Drosophila* embryo. The Bicoid gradient provides biologists with an exceptional system to study morphogens, as the *Drosophila* embryo develops in a syncytium, allowing simple diffusion to generate a protein gradient (Driever and Nusslein-Volhard 1988a; Struhl *et al*. 1989; Houchmandzadeh *et al*. 2002; Crauk and Dostatni 2005; Gregor *et al*. 2005). Thus instead of initiating a signaling cascade that must penetrate the cell membrane to impart an effect, Bicoid is a transcription factor that can easily diffuse within the syncytial environment of the early embryo. This feature allows the signal to function quickly in controlling cell fate decisions during development.

bcd is expressed maternally and its mRNA is localized to the anteriormost region of the oocyte. Upon fertilization of the embryo, *bcd* mRNA is translated and the protein product forms an anterior to posterior gradient (Driever and Nusslein-Volhard 1988a). $bcd^{-/-}$ mutant mothers produce embryos that lack all anterior structures including the head and thorax, consistent with Bcd's role in anterior patterning, and instead develop posterior structures at the anterior of the embryo (Frohnhofer *et al*. 1986). As a result of the morphogenetic properties of the Bcd gradient, adjusting levels of the protein results in shifts in the fate map of the embryo. For example, reducing *bcd* gene dosage results in an anterior shift of the embryonic head fold. Moreover, an increase in gene dosage results in the posterior shift of the head fold, as well as posterior shift of expression of all stripes of the pair rule gene *eve* (Driever and Nusslein-Volhard 1988b).

Once a gradient has been established, cells along the AP axis must interpret the positional and functional information encoded within the Bcd morphogenetic gradient. The strategy employed by the early *Drosophila* embryo involves the differential affinities of Bcd for binding sites present within the enhancers that regulate expression of target genes. For example, the gene *hunchback* (*hb*) contains high affinity Bcd binding sites that allow *hb* expression in more posterior regions of the embryo (**Fig. 1**) (Driever *et al*. 1989; Struhl *et al*. 1989). The target gene *orthodenticle* (*otd*), on the other hand, is less sensitive to the Bcd gradient and contains low affinity binding sites that drive expression in a narrow region of the anterior of the embryo, which contains the highest levels of Bcd protein (**Fig. 1**) (Driever *et al*. 1989). It has also become apparent that although the positional information supplied by morphogenetic gradients is indeed indispensable, often combinatorial inputs are necessary to impart precise positional information and sufficient information for cells to make appropriate cell fate decisions (Ochoa-Espinosa *et al*. 2005).

BCD FUNCTIONS AS A TRANSLATIONAL REPRESSOR

In addition to Bcd's role in directing anterior patterning through the activation of downstream targets, Bcd also functions by inhibiting posterior patterning at the anterior by acting as a translational repressor. The maternal gene *cad* is involved in posterior patterning and its mRNA is present homogeneously in the early embryo. Cad translation is repressed at the anterior of the embryo by Bcd binding to a 120 base pair region in the *cad* 3'UTR, termed the Bicoid Response Element (BRE). This ensures that Cad protein is excluded from the anterior of the embryo, in turn creating a posterior to anterior gradient of the Cad protein (Dubnau and Struhl 1996; Rivera-Pomar *et al*. 1996; Niessing *et al*. 1999, 2000, 2002).

THE EVOLUTION OF BCD

Although *bcd* is the anterior patterning center with multiple indispensable functions during *Drosophila* embryogenesis, it has become clear that *bcd* is not a conserved feature of insect embryogenesis and is not found outside members of a highly derived group within the dipteran lineage (Lynch and Desplan 2003a, 2003b). *bcd* is located in a rapidly evolving region of the genome within the homeotic gene complex (Hox-C), adjacent to the gene *zerknullt* (*zen*), the insect *Hox3* homolog. It is thought that despite a great deal of sequence divergence between the two genes, *bcd* arose as a duplication of *zen* (Dearden and Akam 1999; Stauber *et al*. 1999). In *Drosophila*, *zen* is expressed in the dorsal region of the embryo corresponding to the extraembryonic membranes. Interestingly, a study revealed that in certain non-Cyclorrhaphan flies, such as *Empis livida, Haematopota pluvialis* and *Clogmia albipunctata*, the *Hox3* genes show a greater sequence similarity to *zen*. The expression patterns of these genes however, show characteristics of both the anterior *bcd* expression, as well as the dorsal *zen* expression. These findings support the idea that *bcd* evolved as a duplication of *zen* and took on some of the maternal aspects of *zen* expression, whereas *zen* evolved to be restricted to zygotic patterning of the dorsal extraembryonic membranes (Stauber *et al*. 2002). The Bcd homeodomain then acquired the same DNA binding specificity as that of Otd, imparted by the presence of a lysine residue at position 50 of the homeodomain (Treisman *et al*. 1989). Otd's function in patterning anterior embryonic regions is highly conserved from the most primitive animals to vertebrates. This new feature allowed Bcd to regulate many of Otd's target genes, replacing Otd as the major player of anterior development in the embryonic patterning network (Lynch and Desplan 2003a, 2003b).

ANCESTRAL ANTERIOR PATTERNING FACTORS: HB AND OTD

As it became apparent that *bcd* is a unique addition to the developmental network in *Drosophila*, ancestral characters responsible for early insect patterning were identified (Schroder 2003). It was hypothesized that current conserved downstream targets of Bcd might play the role of an anterior patterning center in more ancestral insects. The primary candidates to function ancestrally in a *bcd*-like manner were *hb* and *otd* (Lynch and Desplan 2003a, 2003b).

hb is expressed maternally throughout the entire length of the *Drosophila* embryo. The Hb protein forms an anterior to posterior gradient as a result of translational repression in the posterior of the embryo by the maternal factor Nanos (Nos) (Lehmann and Nusslein-Volhard 1991). *hb* is later expressed zygotically at the anterior of the embryo in a Bcd-dependent manner (Driever and Nusslein-Volhard 1989; Driever *et al*. 1989; Struhl *et al*. 1989).

The maternal component of *hb* seems non-essential, as embryos lacking maternal *hb* do not exhibit a phenotype: maternal *hb* might instead play a redundant role with Bcd in patterning the anterior (Hulskamp *et al*. 1989; Irish *et al*. 1989; Lynch and Desplan 2003a; Pultz *et al*. 2005). However, the role of hb^{mat} becomes evident in embryos doubly mutant for *bcd* and hb^{mat}, which have a reversed polarity resulting in "bicaudal" embryos. This phenotype is more severe than the *bcd* phenotype alone where, although the telson is duplicated at the anterior, all remaining segments show normal polarity (Gavis and Lehmann 1992). Moreover, simultaneous removal of both maternal and zygotic *hb* components results in embryos resembling the bicaudal phenotype seen with removal of both hb^{mat} and *bcd* (Simpson-Brose *et al*. 1994). Embryos homozygous for zygotic *hb* lack labial and thoracic segments, and have a fusion of abdominal segments 7 and 8 (A_7–A_8) (Lehmann and Nusslein-Volhard 1987). Taken together, these results suggest that despite *bcd*'s critical role in anterior patterning, *hb* is also essential for early anterior patterning in the *Drosophila* embryo. Elegant experiments have further shown that hb^{mat} can partly replace some of *bcd* function. For example, Bcd's role in activating zygotic *hunchback* can be made obsolete. Embryos with an increased dosage of hb^{mat} in the absence of *bcd* still develop some thoracic segments (Wimmer *et al*. 2000).

Another conserved downstream target of Bcd involved in patterning the ocular and antennal segments in *Drosophila* is *otd* (Cohen and Jurgens 1990). Both Otd and Bcd are homeodomain transcription factors that contain a lysine at position 50 of their homeodomains. This critical residue gives both these factors the same binding specificity, thus allowing both proteins to share targets and possibly patterning functions (Treisman *et al*. 1989).

The hypothesis that Hb and Otd might function together ancestrally to achieve anterior patterning in the absence of Bcd has now been thoroughly tested in both *Tribolium* and *Nasonia* (Schroder 2003; Lynch *et al*. 2006a). In contrast to the solely zygotic *otd* expression in *Drosophila*, *Tribolium otd-1* is expressed maternally in the anterior of the developing embryo. Using parental RNA interference (pRNAi) to knock down the function of *otd-1* in *Tribolium* results in loss of head structures. Conversely, knock down of *Tribolium hb* via pRNAi results in loss of thoracic and gnathal structures. In the absence of both *hb* and *otd-1*, however, the head, thorax and anterior abdomen of the beetle do not form. This phenotype is strikingly similar to that reported for strong *bcd* mutant embryos (Schroder 2003).

NASONIA AS A MODEL SYSTEM

The realization that the well understood genetic network and mechanism of embryogenesis of *Drosophila* is in fact highly derived, spurred many researchers within the field of evolution and development to decipher the true ancestral network and mechanisms driving insect embryogenesis. As work in a number of short germ insects has highlighted both common and divergent features within embryogenesis, it has become increasingly apparent that a greater understanding of long germ development is crucial to understanding the evolution of insect embryogenesis from short to long modes. As a result, developmental biologists have sought out other long germ insects to study the common features of this mode of embryogenesis in relation to what is known in *Drosophila*. *Nasonia vitripennis* was recently established as a model system to explore these questions (Pultz *et al*. 1999, 2000, 2005; Lynch 2005; Lynch *et al*. 2006a, 2006b; Olesnicky *et al*. 2006; Brent AE 2007). *Nasonia* is a long germ embryo that lacks the anterior patterning gene *bcd* (Lynch *et al*. 2006a). Moreover, recent evidence suggests that long germ development among Hymenopterans (*Nasonia)* and Dipterans was derived independently (Savard *et al*. 2006). This system, therefore gives us an opportunity to study the result of convergent evolution within the developing insect embryo.

Nasonia is a member of the Family Chalcidae and the genus is comprised of three species of wasps that are para-

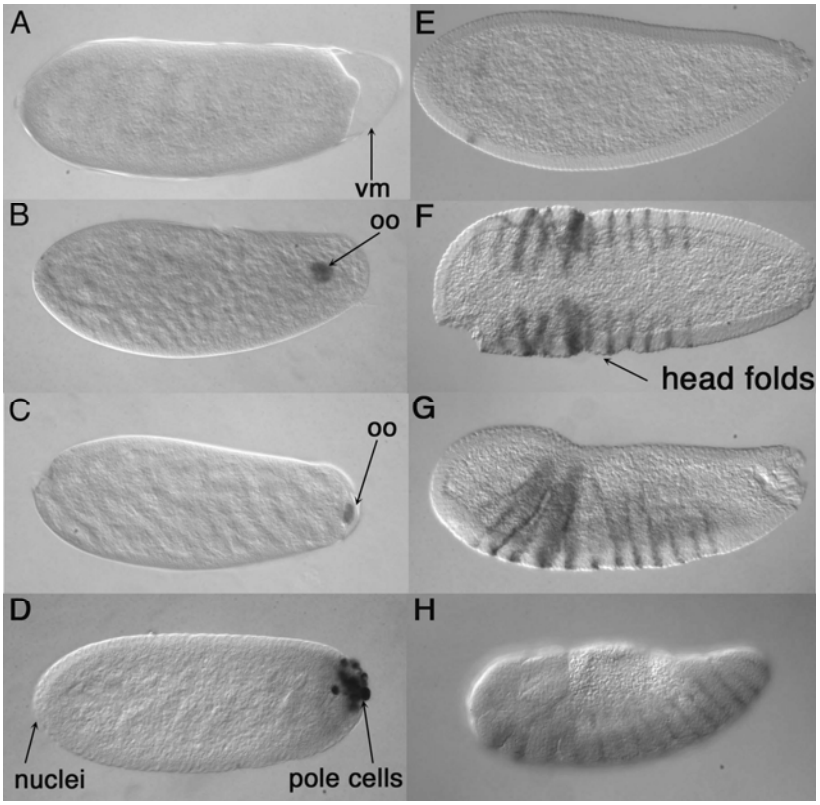

Fig. 2 Development of the *Nasonia* embryo. A clear vitelline membrane (vm) surrounds the embryo (**A**). The oosome (oo) is stained for *otd* mRNA (**B**). The oosome migrates to the posterior of the embryo (**C**). Pole cells (stained for *nos* mRNA) bud off from the posterior pole as nuclei migrate to the periphery of the embryo (**D**). *en* is turned on first at the anterior in the blastoderm embryo (**E**). *en* is activated in an anterior to posterior progression and head folds become visible at the onset of gastrulation (**F**, dorsal view, **G**, lateral view). All *en* stripes, and thus segments become visible by the end of germ band extension (**H**).

sitoids of large fly's pupae: *Nasonia vitripennis*, *Nasonia girault* and *Nasonia longicornis* (Campbell *et al*. 1993). *N. vitripennis* is found throughout the world and has been used both commercially in pest control of larger flies and academically for genetic and developmental research (Werren *et al*. 1986; Pultz and Leaf 2003; Beukeboom *et al*. 2007). The genome size of *Nasonia* is approximately 335 Megabases in five chromosomes, as opposed to *Drosophila* whose genome is 180 Megabases in four chromosomes. Despite the fact that *Nasonia*, a Hymenopteran, and Diptera are separated by over 200 million years of evolution, *Nasonia* and *Drosophila* share many similar characteristics in their modes of embryogenesis, making these species ideal for studying the (convergent) characteristics of long germ development (Pultz and Leaf 2003).

Nasonia is easily maintained in the laboratory and has a life span comparable to *Drosophila*. This wasp provides a unique system for the study of zygotic lethal mutations because of the ability to apply haplo-diploid genetics to the system (Pultz *et al*. 1999, 2000, 2005; Olesnicky *et al*. 2006). Females deposit eggs inside the pupae of large flies and fertilized eggs develop into females, while unfertilized eggs will develop as males, facilitating screening for zygotic lethal mutations in the all male progeny of unmated females (Pultz *et al*. 2000; Werren and Stouthamer 2003; Beukeboom *et al*. 2007). Also, although the life cycle is completed in approximately 2 weeks at 28°C, progeny will enter diapause if mothers are raised in severe conditions, such that they can be stored at 4°C for up to 16 months and will resume development once returned to room temperature (Perrot-Minnot *et al*. 1996; Pultz and Leaf 2003). Thus, the ease of handling *Nasonia*, as well as screening for zygotic lethal mutations, has led to the emergence of this parasitoid wasp as a model system for the study of a variety of questions including behavior, sex determination, wing size and speciation (Pultz and Leaf 2003).

THE *NASONIA VITRIPENNIS* LIFE CYCLE

Nasonia vitripennis is a parasitoid wasp that completes its embryonic and larval development within a single host pupa. The *Nasonia* larvae and adults feed on host pupal hemolymph, although adults can also feed on sugar water and honey. Adult female wasps lay eggs in the pupa of larger flies by drilling a hole into the host pupal case using their ovipositor. The female wasp then injects the host with venom that arrests pupal development. The female deposits her eggs on the surface of the host pupa, where the embryos will undergo embryogenesis. The female, if left unmated, will produce only male progeny. When the female has mated, she is able to control the sex ratio of her clutch depending on the environmental conditions and can lay both females and males. Under favorable conditions, a female will lay a clutch of between 20 and 35 embryos per host pupa (Davies and King 1972; Parker and Orzack 1985).

NASONIA EMBRYOGENESIS

The *Nasonia* embryo is covered by a smooth and flexible chorion and a vitelline membrane, both of which contain no visible structures. Both layers are clear and allow for observation of embryogenesis through a dissecting microscope (**Fig. 2A**). At egg deposition, the embryo is arrested in meiosis I. As meiosis completes, the cytoplasm at both anterior and posterior poles clears in contrast to the more densely stained cytoplasm at the center of the embryo (Pultz and Leaf 2003). Additionally, the oosome, a structure containing the germplasm, becomes evident at the posterior of the embryo (**Fig. 2B, 2C**). One hour after the completion of meiosis I and the fusion of pronuclei in fertilized embryos, the nuclei enter into the first mitosis and undergo a series of synchronous divisions, resulting in the formation of a syncytial embryo (Tram and Sullivan 2000; Pultz and Leaf 2003). After the eighth nuclear division (~3 hours after egg deposition at 25°C), pole cells begin to bud off from the posterior pole of the embryo (**Fig. 2D**). The formation of pole cells coincides with the migration of embryonic nuclei to the periphery of the embryo. Here the nuclei will continue to divide until cellularization, when membranes are laid down around the nuclei to form individual cells. As cellularization proceeds (~7 hours after egg depostion at 25°C), the embryo becomes narrower, elongating at the posterior pole, with an obvious thickening along the periphery of the embryo, making a physical separation from the yolky center of the embryo. Gastrulation occurs at approximately 10 hours after egg deposition at 25°C and is easily identified

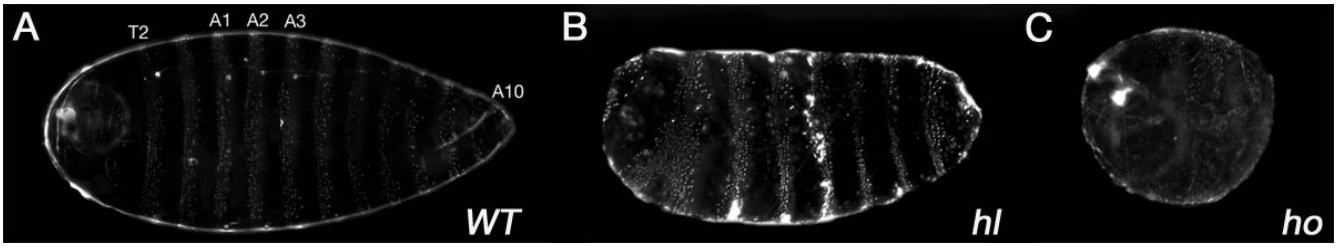

Fig. 3 A genetic screen for patterning mutants in *Nasonia*. Wild type cuticle composed of head segments, 3 thoracic segments and 10 abdominal segments (**A**). *headless* (*hunchback*) cuticle shows thoracic and head defects (**B**). *head only* cuticle shows loss of abdomen and posterior structures (**C**).

by the formation of folds at the anterior of the embryo, corresponding to the gnathal head region (**Fig. 2F**). The germ band begins to extend and segments become apparent in an anterior to posterior progression (**Fig. 2G, 2H**). After 30 hours at 25°C, the *Nasonia* larva hatches (reviewed by Pultz and Leaf 2003).

A SCREEN FOR ZYGOTIC EMBRYONIC PATTERNING MUTANTS

In order to appropriately compare the developmental programs of *Drosophila* and *Nasonia,* a genetic screen for the identification of zygotic patterning mutants was conducted by Pultz *et al*. (1999). The screen was designed to identify the major players involved in AP axis patterning, similar to the saturation screens carried out by Nusslein-Volhard and Wieschaus (1980) in *Drosophila*. In the *Nasonia* screen*,* approximately 100 embryonic patterning mutants were identified including mutants that resemble the phenotypes associated with mutations in *Drosophila* genes of the axial-patterning, gap, pair-rule and Polycomb groups. A number of novel mutant phenotypes that do not resemble any known *Drosophila* mutants were also isolated. Mutations were characterized based on cuticular patterns, as well as altered expression of *engrailed* and the thoracic HOX genes *Ultrabithorax* and *Abdominal-A*. In wild type cuticles of *Nasonia* first instar larvae, there are distinct denticle belts for the three thoracic and ten abdominal segments. Furthermore, there are large spiracles on the second thoracic segment, as well as the first three abdominal segments making their identification straightforward (**Fig. 3A**) (Pultz *et al*. 1999, 2000). Overall, the screen showed that zygotic mutations in *Nasonia* often create much more severe patterning phenotypes than those observed for any *Drosophila* zygotic mutants (**Fig. 3B, 3C**). This suggested that *Nasonia* relies more on zygotic patterning input for axis patterning than on maternal function (Pultz *et al*. 2000), as is the case for *Drosophila* (Pultz *et al*. 1999; Pultz and Leaf 2003). Alternatively, the severity of the zygotic phenotypes may be due to an inability of maternal genes to rescue loss of their zygotic counterparts, as their expression patterns do not overlap temporally. This may be due to developmental timing as pre-gastrulation events in *Nasonia* take approximately 10 hours compared to the 3 hours in *Drosophila* (Olesnicky *et al*. 2006).

The success of the *Nasonia* genetic screen and its impact within the field of evolution and development became apparent with the isolation of the zygotic *Nasonia hunchback* null allele, *headless*. Pultz *et al*. (2005) identified a deletion spanning 1.5 kb in the *hunchback* locus responsible for the severe *headless* mutant phenotype (**Fig. 3B**). Loss of zygotic *Nv hb* results in loss of most of the head and the entire thorax, whereas the loss of zygotic *hb* in *Drosophila* is much less severe due to the ability of maternal *Dm hb* to rescue patterning of anterior structures. These results suggest that the wasp cannot compensate for loss of zygotic function with maternal contributions as early development is much longer than in flies and the maternal supply is exhausted before zygotic gene expression is turned on (Pultz *et al*. 2005).

The development of parental RNA interference (pRNAi) in *Nasonia* has facilitated the study of both maternal and zygotic factors involved in early embryonic development (Lynch 2005; Lynch *et al*. 2006a, 2006b; Brent AE 2007). Lynch *et al*. (2006) provide striking evidence in support of the long-standing theory that *otd* and *hb* function ancestrally to replace the role of *bcd* in anterior patterning. Additionally, work by Olesnicky *et al*. (2006) using pRNAi and genetic mutant analysis shows that the posterior patterning gene *caudal* (*cad)* acts as a posterior patterning center, and in conjunction with Otd-1 patterns the entire abdomen of the wasp embryo (**Fig. 4**).

OTD-1 FUNCTIONS AS A MORPHOGEN IN THE *NASONIA* EMBRYO

In the long germ insect *Nasonia*, *otd-1* mRNA is maternally expressed and localized to both poles of the oocyte during oogenesis (**Fig. 5A, 5B**) (Lynch *et al*. 2006). During early embryogenesis, Otd-1 protein is first made at the anterior

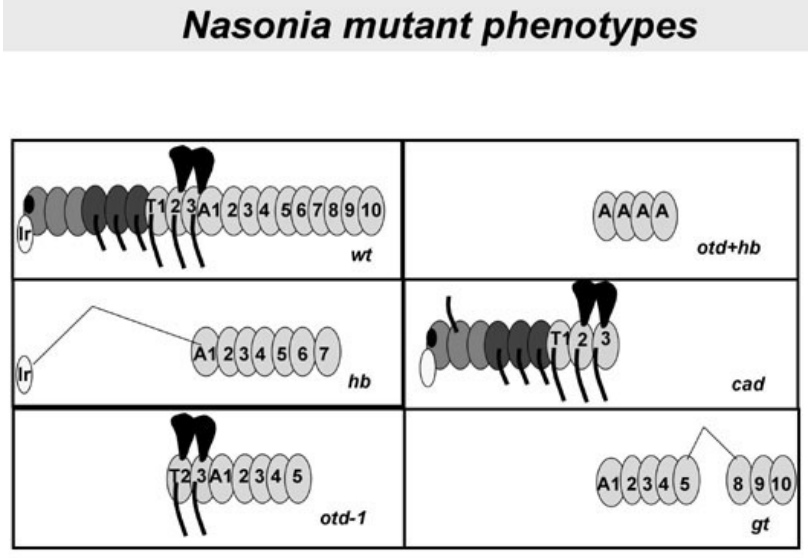

Fig. 4 Summary of *Nasonia* segmentation phenotypes. The wild type Nasonia body plan is composed of head segments (including the labrum (lr), 3 thoracic segments (T1-T3) and 10 abdominal segments (A1-A10). Loss of *hb* results in loss of head and thoracic segments, as well as posterior defects (A8-A10). The labrum, however, is patterned in the absence of *hb*. *otd-1* knockdown results in loss of all head segments, in addition to loss of posterior abdominal segments. Simultaneous knockdown of *otd-1* and *hb* results in loss of all head and thoracic segments, as well as loss of some abdominal segments. This phenotype is reminiscent of *Drosophila bcd* mutant embryos. *cad* knockdown results in the loss of all abdomi-nal segments, pointing to its role as a posterior patterning center. Finally loss of *gt* via parental RNAi results in a severe phenotype where all head and thoracic structures are missing, in addition to the fusion or deletion of A6 and A7.

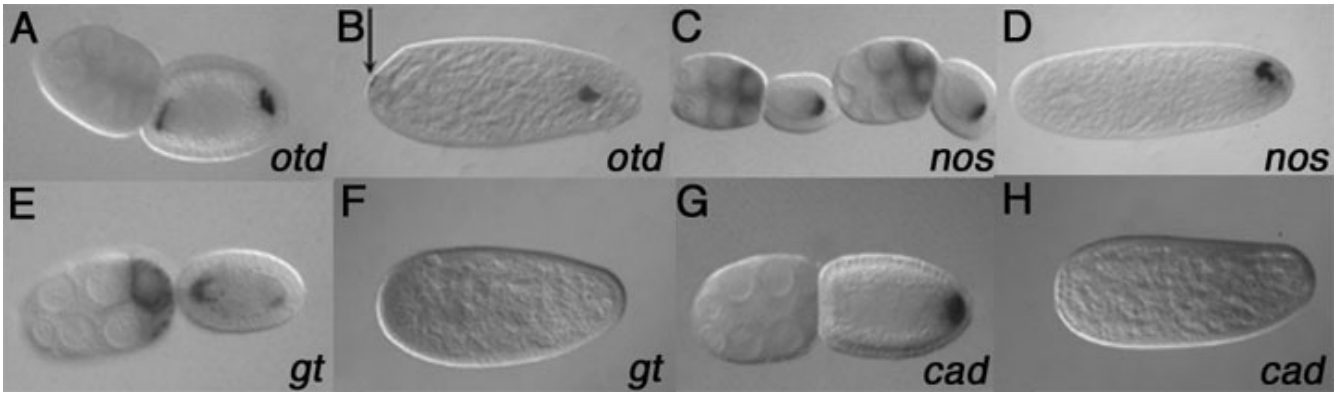

Fig. 5 mRNA localization of maternal factors is used extensively in *Nasonia* development. The *Nasonia* follicle consists of 15 nurse cells separated by a constriction from the oocyte. Maternal mRNAs are made in the nurse cells and transported into the oocyte during oogenesis. *otd-1* mRNA is localized to both the anterior and posterior poles of the *Nasonia* oocyte during oogenesis (**A**). In freshly laid embryos this localization is also seen in the anterior (see arrow) and in the posterior, where *otd-1* localizes to the germ plasm containing structure, termed the oosome (**B**). *nos* mRNA is localized to the posterior of the developing oocyte (**C**). In the early embryo, this localization is maintained within the oosome (**D**). In *Nasonia*, *gt* is expressed maternally in the nurse cells and transported to the oocyte where it is localized to the oocyte nucleus at the dorsal anterior of the oocyte (**E**). Once the embryo is laid, *gt* mRNA forms a posterior to anterior mRNA gradient (**F**). *cad* mRNA is localized to the posterior of the oocyte during oogenesis (**G**). This localization breaks down such that freshly laid embryos form a posterior to anterior *cad* mRNA gradient (**H**).

pole and translationally repressed at the posterior of the embryo. The presence of *nanos response elements* in the *otd-1* 3'UTR suggests that Nos protein functions to prevent Otd-1 (and Hb) translation at the posterior of the embryo, similar to its role as a translational repressor of Hb in the posterior of the *Drosophila* embryo (Hulskamp *et al*. 1989; Irish *et al*. 1989; Sonoda and Wharton 1999; Lynch *et al*. 2006a). Eventually, Otd-1 protein gradients form at both poles. Knockdown of *otd-1* expression via pRNAi results in a shift of target gene expression towards the poles of the embryo, consistent with *otd-1* functioning as a morphogen at both poles. Specifically, the anterior patterning gap gene *empty spiracles* (*ems*) is shifted farther towards the anterior pole in response to increased strength of *otd-1* pRNAi. Both the anterior and posterior expression domains of the gap genes *giant* (*gt*) and zygotic *hb* are also shifted towards the poles in response to *otd-1* knockdown. The molecular phenotypes resulting from *otd-1* pRNAi show that Otd-1 functions as a morphogen in the wasp, similar to the way Bcd functions in the fly (Lynch *et al*. 2006a).

The cuticular phenotype resulting from *otd-1* knockdown results in loss of head segments, as well as loss of posteriormost abdominal segments (**Fig. 4**). This phenotype is much more severe than that of loss of the zygotic gap gene *Dm otd*. Although the *otd-1* knockdown phenotype is not identical to the $bcd^{-/-}$ mutant phenotype, which also lacks thoracic segments (Frohnhofer *et al*. 1986; Cohen and Jurgens 1990), these results do further confirm the hypothesis that Otd, at least in part, functions ancestrally as a major anterior determinant in the absence of Bcd. Moreover, as both Bcd and Otd have the same binding specificity, they likely share similar downstream targets (Treisman *et al*. 1989; Lynch *et al*. 2006a).

hb IS A CONSERVED ANTERIOR PATTERNING GENE IN *NASONIA*

The zinc finger transcription factor *hb* is expressed maternally and zygotically in the *Nasonia* embryo. Maternal *hb* is first transcribed during oogenesis in the nurse cells and loaded throughout the oocyte. In the early embryo, *hb* mRNA is homogenously present throughout the entire length of the embryo. Upon translation of the maternal *hb* mRNA, Hb protein creates an anterior to posterior gradient in the early embryo (Pultz *et al*. 2005). The presence of multiple sequences resembling *nanos response elements* (NREs) in the *Nvit hb* 3'UTR suggests that the *hb* mRNA is translationally repressed in the posterior of the embryo by *nanos*, as is *Dm hb* in *Drosophila* (Hulskamp *et al*. 1989; Irish *et al*. 1989; Struhl 1989; Murata and Wharton 1995; Pultz *et al*. 2005).

At the anterior of the embryo, zygotic *hb* forms a large cap of expression that eventually fades to form a stripe, similar to the zygotic anterior *Dm hb* expression (**Fig. 1G**, **1H**). Zygotic *hb* is also expressed in a stripe at the posterior of the embryo and as a dorsal strip corresponding to the extraembryonic membranes (Pultz *et al*. 2005). Using the $hb^{headless}$ mutant wasp strain, a null allele of zygotic *hb*, Pultz *et al*. (1999, 2005) examined the phenotype resulting from loss of zygotic *hb* in the wasp embryo (Pultz *et al*. 1999, 2000, 2005). Cuticles show both posterior and thoracic defects as expected from the zygotic *hb* expression pattern. Specifically, the posterior abdominal segments A8-A10 are missing and thoracic, gnathal and antennal segments in the head region are defective or missing. Labral structures, however, are present, suggesting that zygotic *hb* does not function alone in anterior patterning (**Fig. 4**). It is also clear that *Nasonia* relies more heavily on zygotic *hb* than the fly, whose loss leads to less severe cuticular phenotypes (Pultz *et al*. 1999). This may be due to redundant functions of Bcd and Hb in flies. Conversely, the greater reliance on zygotic *hb* in the wasp embryo may be a reflection of the lack of *bcd* during wasp embryogenesis.

OTD-1 AND HB FUNCTION SYNERGISTICALLY TO PATTERN THE ANTERIOR OF THE WASP EMBRYO

otd-1 knockdown in *Nasonia* results in loss of head segments, as well as loss of posterior segments. Knocking down both *hb* and *otd-1*, however, results in a complete loss of head and thorax in addition to loss of posterior structures. The anterior segmentation phenotype is more severe than the combination of the individual pRNAi knockdowns of either *hb* or *otd-1*, suggesting that these genes work synergistically in the anterior of the embryo (**Fig. 4**). There is no evidence for a synergistic relationship between *hb* and *otd-1*, however, in patterning the posterior of the wasp embryo (Lynch *et al*. 2006a). This study, in conjunction with the investigation of anterior patterning in the beetle (Schroder 2003) provide strong support for the hypothesis that Hb and Otd play the ancestral role of Bcd in patterning the anterior of the embryo.

MATERNAL GIANT IS A KEY REPRESSOR IN THE ANTERIOR OF THE WASP EMBRYO

Although many basic mechanisms in embryogenesis are conserved throughout the insect world, a common theme that has emerged as a result of many recently reported com-

parative studies is that many members within the genetic network of early embryogenesis seem to take on new roles throughout evolution. This is exemplified by the recent report regarding the expression and function of *giant* (*gt*) in the wasp embryo (Brent *et al*. 2007). In *Drosophila*, *gt* is a zygotic gap gene that is expressed in a *bcd* activated anterior stripe, as well as in a posterior stripe (Kraut and Levine 1991b). In *Nasonia*, the expression of this zygotic gap gene is very similar to its *Drosophila* counterpart (**Fig. 1I, 1J**) (Olesnicky 2006; Brent *et al*. 2007). Strikingly, however, freshly laid wasp embryos show an anterior to posterior *gt* mRNA gradient, indicating that *gt* has taken on a new role as a maternal determinant during wasp embryogenesis (Brent *et al*. 2007). In fact, follicles harvested from female wasps show that *gt* mRNA is tightly localized to the oocyte nucleus in the anterior of the developing oocyte (**Fig. 5E**, **5F**). This again highlights the use among insects of conserved mechanisms, such as mRNA localization, and the adaptation of these strategies to create novel roles for existing genes within the genetic network.

The evolution of *gt* as a maternally expressed factor in *Nasonia* suggests a new function for *gt* during development. *gt* pRNAi embryos show loss of all head and thoracic segments, as well as fusion or deletion of abdominal segments 6 and 7, likely due to loss of the posterior zygotic *gt* domain (**Fig. 4**). Therefore, *Nasonia gt* has gained a new essential role in head and thoracic patterning resulting in a phenotype much more severe than the mild head phenotype of *Dm gt* mutants, which only show loss of labral and labial structures (Kraut and Levine 1991b). An elegant molecular analysis by Brent *et al*. (2007) on the effect on the genetic network resulting from loss of *gt* ascribed a major role for *gt* as a potent repressor of *Kr* in the anterior of the wasp embryo. Thus, the loss of *gt* results in an expansion of *Kr* to the anterior tip of the developing embryo, preventing the formation of any head or thoracic segments. This study highlights the ever-changing roles of developmental genes throughout evolution and the ability of these plastic genetic networks to pattern a basic segmented body plan that is conserved among insects.

COMBINATORIAL INPUT ALONG THE AP AXIS: BICOID AND CAUDAL

One study in *Drosophila* showed that the combined activities of Bcd and Cad are involved in driving expression of *knirps* (*kni*) in the posterior of the embryo via two response elements in the *kni* regulatory region. This study is of particular interest as *bcd* is an anterior determinant, yet here it plays a role in driving expression of a target gene in a more posterior region of the embryo. Moreover, neither Bcd nor Cad alone is sufficient to drive wild type expression of *kni* in the embryo (Rivera-Pomar *et al*. 1995). Another example of a target gene responding to the reciprocal gradients of the transcription factors Bcd and Cad, is *hairy* (*h*), a pair-rule gene expressed in 7 stripes in the embryo. Similar to *even skipped* (*eve*), the elements driving *h* stripes can be divided into separate modules. In the case of *h* stripe 7, the most posteriorly expressed *h* stripe, a number of Bcd and Cad sites are necessary to drive wild type expression. Furthermore, removal of both *cad* and *bcd* results in a severe reduction in *h* stripe 7 expression (La Rosee *et al*. 1997).

Using the computer algorithm Ahab, a recent study utilized known binding sites of various maternal and gap genes, as well as equilibrium thermodynamics to recover new segmentation elements in the regulatory regions of segmentation genes (Schroeder *et al*. 2004). The expression patterns of these elements revealed that genes expressed in the anterior of the embryo showed a great number of Bcd binding sites, whereas Cad binding sites were few. Conversely, elements expressed in the posterior half of the embryo tended to have a greater number of Cad sites and few Bcd binding sites. Furthermore, the elements expressed early in the embryo contained binding sites for a number of maternal factors including Bcd and Cad, strongly suggesting that these maternal factors directly control most of early zygotic patterning (Schroeder *et al*. 2004).

CAD MAY BE A POSTERIOR PATTERNING CENTER IN SHORT GERM EMBRYOS

It has been proposed that an anterior patterning center, such as Bcd in the long germ *Drosophila*, would not function well to pattern the anterior of the embryo in short germ insects (Stauber *et al*. 1999; Stauber *et al*. 2002): Anteriorly localized factors would not be able to reach the germ rudiment at the posterior of the oocyte and would instead pattern the extraembryonic membranes, which lie at the anterior (Lall and Patel 2001; van der Zee *et al*. 2005). Instead, the ancestral system may have exclusively utilized a posterior patterning center, allowing for posteriorly localized factors to easily reach the developing embryo. One candidate for an ancestral posterior patterning function is *cad*. In both *Tribolium* and *Gryllus, cad* is expressed early in a broad posterior to anterior gradient and it appears to regulate expression of anterior *hb* and *Kr* placing it atop the genetic patterning hierarchy (Copf *et al*. 2003; Shinmyo *et al*. 2005).

CAD IS A POSTERIOR PATTERNING CENTER IN THE *NASONIA* EMBRYO

Among the various mutant phenotypes isolated in the Pultz *et al* screen, *head only* (*ho*) was shown to have dramatic posterior patterning defects that partly overlap with the posterior cuticular phenotypes derived from knockdown of *otd*. *ho* is a mutant that develops only head and thorax, lacking all abdominal and posterior structures (**Fig. 3C**). Comparison of the *ho* phenotype to known *Drosophila* mutant phenotypes strongly suggested that *ho* is due to a lesion in the *Nasonia cad* locus. Moreover, *cad* pRNAi phenocopies the *ho* mutant phenotype and genetic mapping experiments place the *ho* genetic lesion very close to the *Nv cad* locus (**Fig. 4**) (Olesnicky *et al*. 2006). Similar to the phenotype resulting from loss of zygotic *Nv hb*, however, *ho* results in a much more dramatic phenotype than loss of zygotic *cad* alone in *Drosophila* and instead resembles loss of both maternal and zygotic *Dm cad*. This strongly suggests that the wasp needs both genetic components during embryogenesis (Pultz *et al*. 1999).

In *Nasonia*, *cad* is expressed both maternally and zygotically. Expression analysis of ovarian follicles and early embryos uncovered a surprising mechanism for the generation of the maternal Cad gradient in the wasp embryo (Olesnicky *et al*. 2006). In *Drosophila*, *Tribolium* and even *C. elegans*, formation of the Cad gradient is achieved through translational repression of *cad* mRNA in the anterior of the embryo (Macdonald and Struhl 1986; Mlodzik and Gehring 1987; Hunter and Kenyon 1996; Schulz *et al*. 1998). In the fly, Bcd functions as the Cad translational repressor by binding to the *cad* 3'UTR (Dubnau and Struhl 1996; Rivera-Pomar *et al*. 1996; Niessing *et al*. 1999, 2000, 2002). While the factors involved in establishing the Cad gradient in *Tribolium* are not known, in *C. elegans*, a factor completely unrelated to Bcd, *mex-3*, serves as a translational repressor of the *cad* homolog *pal-1* (Hunter and Kenyon 1996). In *Nasonia*, *cad* mRNA is instead localized to the posterior pole of the oocyte during oogenesis and forms a posterior to anterior mRNA gradient by simple diffusion from the posterior pole (**Fig. 5G, 5H**). This result is particularly interesting because the use of mRNA localization and diffusion obviates the need for translational repression at the anterior of the embryo to efficiently establish a Cad gradient (Olesnicky *et al*. 2006).

Zygotic *cad* is expressed in the posterior two thirds of the early embryo and begins to recede from the anterior as embryogenesis progresses. Later, *cad* is expressed as two stripes in the posterior, and finally in a single stripe at gastrulation. Functional analysis of *cad* using pRNAi showed that maternal *Nv* Cad is a major transcriptional regulator of

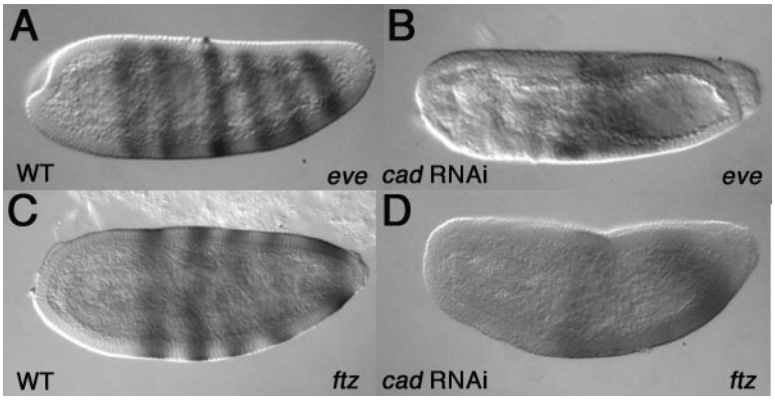

Fig. 6 *cad* knockdown results in aberrant pair rule gene expression. Wild type *eve* in *Nasonia* is expressed in an anterior to posterior progression as stripes along the antero-posterior axis. Secondary pair rule stripes can be seen forming from the anteriormost stripes (**A**). Loss of *cad* via pRNAi results in severe defects including the formation of tumor-like structures at the posterior. Additionally, anterior *eve* expression is initiated as one broad stripe but does not resolve into a normal striped pattern (**B**). Wild type *ftz* is expressed in 6 stripes along the antero-posterior axis (**C**). *cad* knockdown results in loss of most *ftz* stripes (**D**).

genes expressed in the thoracic and abdominal regions of the embryo, whereas *Dm* Cad does not function in thoracic patterning. For example, in *Nasonia*, Cad is a transcriptional activator of the gene *Kr*, which forms a broad stripe at the center of the embryo and is essential for normal abdominal patterning. Similarly, anterior and posterior *kni* expression is absent in embryos lacking *Nv cad*. Moreover, in the wasp embryo, *cad* plays a role as an activator of *gt* and *tailless* (*tll*). In contrast, removal of both maternal and zygotic *cad* components has no effect on *Kr* expression in *Drosophila* and only functions as a weak activator of posterior *kni* expression. Additionally, by comparing results obtained from *cad* pRNAi mutant embryos and the zygotic *Nv cad* mutant embryos, it becomes clear that maternal *cad* functions in *Nasonia* specifically as an activator of anterior gap and pair rule gene expression, whereas zygotic *cad* functions as an activator in more posterior regions of the wasp embryo (Olesnicky *et al.* 2006).

Thus, *Drosophila* relies much less on *cad* function than *Nasonia* or other insects such as *Tribolium* and *Gryllus* (Copf *et al*. 2004; Shinmyo *et al*. 2005; Olesnicky *et al*. 2006): As the genetic network evolved, Cad progressively lost in flies its importance as an activator of the gap genes. The fact that Bcd, in conjunction with Cad, is also important for the activation of posterior genes in the fly suggests that Bcd may have usurped much of Cad's role as the major posterior patterning gene. Cad does, however retain an important role as a regulator of pair rule gene expression in the posterior half of the fly embryo, maintaining some of its role as a major posterior patterning factor (**Fig. 6A-D**) (Olesnicky *et al*. 2006).

mRNA LOCALIZATION: A COMMON FEATURE OF LONG GERM EMBRYOGENESIS

mRNA localization is commonly used for targeting factors to particular regions of a cell or tissue. In *Drosophila*, mRNA localization of maternal factors is used extensively to set up the antero-posterior axis of the early embryo, prior to the onset of zygotic gap gene expression (St Johnston 2005; Zimyanin *et al.* 2007). In fact, the use of localized mRNAs, such as the anterior patterning factor *bcd*, allows for the generation of steep concentration gradients of proteins through translation and simple diffusion. Thus mRNA localization in a syncytial embryo is a superb mechanism for setting up an environment that utilizes morphogens for patterning.

In *Drosophila*, *bcd* assumed the role of the anterior patterning center and its mRNA is localized maternally to the anteriormost pole of the embryo. Similarly, in the long germ wasp *Nasonia*, *otd-1* is maternally localized to the anterior pole of the early embryo and functions similarly to *bcd* in patterning the anterior segments through a morphogenetic protein gradient. Surprisingly, *otd-1* mRNA is also localized to the posterior pole of the *Nasonia* embryo to help direct posterior patterning (Lynch *et al*. 2006a). In addition to *otd-1*, a number of maternal mRNAs are also localized in the early wasp embryo. *gt* mRNA is expressed maternally and localized to the anterior pole of the developing oocyte (Brent *et al*. 2007). Moreover, *nos* and *cad* mRNAs are localized to the posterior pole of the embryo (**Fig. 5A-H**) (Lynch *et al*. 2006a; Lynch and Desplan submitted). As *Nasonia* undergoes long germ development similar to *Drosophila*, it becomes evident that in addition to a heavy reliance on maternal patterning factors, one common feature of long germ embryogenesis is the importance of proper localization of these maternal determinants.

Recent work by Olesnicky and Desplan (2007) shows that the major mechanisms employed for maternal mRNA localization are common to both *Nasonia* and *Drosophila*. Specifically, transport of mRNAs along the microtubule cytoskeleton is utilized for the localization of most mRNAs within the oocyte. Most posteriorly localized mRNAs, however, exploit the germ plasm to achieve a tight posterior localization that persists much longer than micro-tubule mediated mRNA localization. By using drug treatments to inhibit the polymerization of microtubules, Olesnicky and Desplan also find that *Nasonia* relies heavily on the microtubule cytoskeleton, not only for transport of factors from the nurse cells to the oocyte, but also during oogenesis for oocyte specification. A similar role in oocyte specification has been ascribed to microtubules in *Drosophila* (Pokrywka 1995; Riparbelli *et al.* 2007).

COMMON STRATEGIES USED DURING LONG GERM INSECT EMBRYOGENESIS

The *Drosophila* embryo utilizes many strategies including mRNA localization, gradient formation and mutual repression to create a very fast and efficient developmental program. Other insects undergo development in a much slower manner and utilize very different mechanisms to achieve their adult body plan. One important aspect of *Drosophila* embryogenesis is the evolution of a syncytial embryo that persists even during the beginning stages of zygotic transcription, allowing the specification of the entire antero-posterior axis in the early embryo. This strategy is also employed by many short germ insects, but only for patterning the anterior embryonic structures. In short germ embryos, the abdominal and posterior structures form later within a cellularized environment at the posterior of the embryo in a region termed the growth zone. Thus, the use of a syncytium to pattern the entire length of the embryo is a feature specific to long germ development (Lall and Patel 2001; Davis and Patel 2002; Liu and Kaufman 2005b).

In a syncytial environment, there are no cellular membranes to create a barrier between nuclei, allowing for formation of gradients of transcription factors via simple diffusion and the simultaneous patterning of the whole length of the antero-posterior axis. For example, *Drosophila* establishes gradients of Hb and Bcd at the anterior embryonic pole, while Cad and Nos gradients are formed at the posterior pole, thus establishing the basic asymmetry along the antero-posterior axis in the earliest phase of development (Liu and Kaufman 2005b; St Johnston 2005). Although gradients of extracellular growth factors are also observed in cellularized environments, they must rely on additional factors to transduce the signal within the cell.

Results presented in this review show that a similar strategy of opposing gradients is used in the *Nasonia* embryo, where Hb and Otd create gradients at the anterior of the embryo and Cad and Otd are graded at the posterior of the embryo. In short germ embryos, although gradients are formed in the early embryo, they are incapable of patterning the entire length of the embryo, as abdominal structures develop much later and within a cellularized environment. Thus, in addition to major differences in the embryonic environment between long and short germ insects, there is also a delay in posterior patterning within short germ embryos. Therefore, with the advent of long germ embryogenesis, a temporal shift must have taken place to accommodate simultaneous expression of the anterior and posterior patterning centers, thus allowing patterning along the entire antero-posterior axis (Lynch and Desplan 2003a; Lynch *et al*. 2006a; Olesnicky *et al*. 2006).

The Bcd gradient is formed in the anterior of the embryo and functions in a morphogenetic manner in *Drosophila*. The Bcd protein serves many purposes in development including activation and translational repression. Strikingly, despite its concentration at the anterior tip of the embryo, Bcd is also important for the activation of genes in posterior regions of the embryo. The combinatorial input of Bcd and Cad has been reported to regulate expression of both gap and pair-rule genes in the posterior or the embryo, whereas Hb and Bcd function together at the anterior of the embryo to activate gap and pair rule genes (La Rosee *et al*. 1997; Hader *et al*. 1998).

In *Nasonia,* the use of a morphogen is also extremely important for normal embryonic development. The wasp, however, lacks Bcd and instead establishes an Otd morphogenetic gradient at both poles of the early embryo. Thus, Otd is able to function throughout the length of the embryo in a morphogenetic manner, similar to Bcd in *Drosophila,* yet is positioned very differently from Bcd. Since Otd is expressed at both poles, it is important for cofactors to create asymmetry in the wasp embryo. Hb serves as a cofactor for Otd in patterning the anterior of the embryo, similar to its role with Bcd in *Drosophila* (Lynch *et al*. 2006a). Recent work shows that Cad is an extremely important posterior patterning gene that serves as a cofactor for Otd in posterior embryonic patterning (Olesnicky *et al*. 2006). Thus both Otd in the wasp and Bcd in the fly provide essential patterning information throughout the entire length of the embryo that serves to initiate transcription of zygotic target genes. Both morphogens, however, require cofactors to initiate the proper developmental program in the different regions of the embryo. Additionally, although a similar combinatorial effect is seen between Cad and Otd in *Nasonia*, as compared to that of Cad and Bcd in *Drosophila*, Cad functions more extensively in directing posterior patterning in the *Nasonia* embryo than it does in the fly.

CONCLUSIONS

In conclusion, research in *Nasonia* and *Drosophila* highlights a number of conserved features employed by long germ insects to establish the antero-posterior axis and initiate patterning along this axis. Initially, maternal mRNAs are transcribed by the nurse cells and transported into the developing oocyte. Next, groups of polarized microtubules within in the dynamic cytoskeleton are used as tracks for motors to shuttle mRNAs to various regions of the oocyte. Additionally, the germ plasm is used for the tight localization of mRNAs and other factors to the posterior cortex of the egg. Upon the completion of oogenesis and subsequently egg deposition, these localized mRNAs begin to diffuse and generate opposing gradients within the syncytial embryo. These gradients provide the positional information along the axis that is necessary to initiate the complex genetic program that will establish the adult body plan. Thus the increasing reliance on maternal factors, the utilization of mRNA localization, gradient formation and a syncytial embryo are hallmarks of long germ embryogenesis. Yet the genes that make up the developmental network are plastic and show a remarkable ability to evolve new functions and expression patterns to adapt to the changing mechanisms and environments of the quickly evolving insect world.

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