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Diversity of molecules and mechanisms in establishing insect anterior–posterior polarity

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Anterior–posterior (AP) patterning is an essential process that requires the generation of large amounts of positional information to properly specify many distinct cell fates along the long axis of the insect embryo. While the general molecular basis of this process has long been known in the fly *Drosophila*, detailed understanding of this process is still emerging in other insect species. What is now clear is that this process in extremely labile, and distinct AP patterning programs can exist even within a single species. This review presents recent progress on this topic in an attempt to synthesize the disparate data and provide an outlook on the future of the field.

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Introduction

The anterior–posterior patterning systems of insect embryos have long served as fruitful models for both generating and testing hypotheses relating to basic developmental mechanisms. The morphogen gradient system of *Drosophila melanogaster* has been particularly fruitful, and has spawned a wide ranging research program trying to understand AP patterning throughout insects [1].

Both anterior and posterior patterning centers are found in the fly embryo. mRNA for the transcription factor Bicoid (Bcd) is localized to the oocyte anterior pole during oogenesis [2], leading to a graded distribution of the protein [3]. This protein gradient, in cooperation with Hunchback (Hb), another maternal transcription factor, regulates target genes in a concentration related manner, and provides most of the AP polarity to the embryo [4–6]. Bcd also has the capability to repress the translation of the ubiquitous maternal mRNA of the posterior factor, Caudal (Cad) [7]. At the posterior center, Nanos (Nos) is required to allow abdomen formation, by repressing the translation of *hb* mRNA [2].

In the past few years progress has been made in understanding variety and AP patterning centers in a broad range of insect species. These new discoveries have given important insights into how developmental systems can change in the course of evolution.

Whence Bicoid?

Despite its critical importance in *Drosophila*, *bcd* is not a universal feature of insects. Rather, it is a highly derived Hox gene [8] and is only found in cyclorrhaphan flies [9]. These discoveries raised the question of how AP patterning is carried out in other insect species, and how *bcd* came to be so critical in *Drosophila*.

One hypothesis was based on the DNA binding properties of Bicoid. Position 50 of the homeodomain determines the preferred binding specificity of homeobox proteins and most Hox-like transcription factors have a glutatmine (Q) at this position [10]. Bcd is unusual among Hox-derived genes in that it has a lysine (K) at position 50, giving it a DNA binding specificity distinct from its Hox ancestors, but identical to distantly related non-Hox K50 homeodomain proteins, such as Orthodenticle (Otd) [10]. If a K50 homeodomain protein played an ancestral AP patterning role, Bcd could have taken advantage of pre-existing enhancers that contained K50 homeodomain binding sites, making the transition to a new anterior center easier than if the enhancers for all AP patterning genes had to change.

Discoveries in the beetle *Tribolium castaneum* (*Tc*), and the wasp *Nasonia vitripennis* (*Nv*) were entirely consistent with the hypothesis of *bicoid* usurping the ancestral anterior morphogen role of *otd* orthologs [11]. In particular, the result in *Nasonia* showed a spectacular case of convergent evolution, as *otd1* mRNA is tightly localized to the anterior pole, similarly to *bcd* [12]. As if to foreshadow the direction of the field, *Nv-otd1* mRNA is also tightly localized to the posterior pole, and has a crucial patterning function there, which was completely unexpected and unprecedented. Indeed, as the analysis of insect AP patterning became both broader phylogenetically and more detailed within favored model species, it became clear that the situation is much more complicated than it might have appeared.

At the anterior, outside of *Drosophila*: a menagerie of molecules and mechanisms

Hymenoptera

In *Nasonia*, a second, unexpected, factor was found to be crucial for anterior patterning in addition to the *bcd*-like activity of *Nv-otd1*. The *Nasonia* ortholog of *giant*, whose *Drosophila* counterpart is a zygotic factor downstream of Bcd, is maternally localized at the anterior, plays a permissive patterning role, and is required for specification of the head and thorax [13]. In the honeybee *Apis mellifera* (*Am*), *giant* is expressed zygotically in a very broad domain, and gives an even more extensive knockdown phenotype [14].

Unlike its *Nasonia* counterpart, *Am-otd1* is not localized in the ovary, but shows an increasing anterior enrichment in the earliest stages of embryogenesis [15••]. RNAi knockdown of *Am-otd1* leads to a massive loss of anterior segments, extending well into the abdomen. This phenotype is more severe than even loss of *bcd* in flies, and much more extensive than the anterior role of *Nv-otd1*. Knockdown of *Am-hb*, whose mRNA is maternally ubiquitous, leads to similar massive loss of anterior structures. This indicates that the cooperation between Otd and Hb may be a general feature of the hymenoptera. Like *Nasonia* and *Tribolium*, there is a second *otd* gene in *Apis*. Unlike the former two species, *Am-otd2* is expressed maternally and has an early patterning function [15••].

Additional differences between *Nasonia* and *Apis* can be seen at the posterior pole. While *Nasonia caudal* (*-cad*) mRNA is localized to the posterior pole (thus obviating the need for the translational repression function of Bcd) [16], *Am-cad* is initially localized to the anterior pole, before relocalizing posteriorly. RNAi against *caudal* causes massive patterning disruption in both species, leading to the loss of all thoracic and abdominal segments in *Nasonia*, and the disorganization and fusion of equivalent segments in *Apis* [14,16].

Finally, it was shown that *nanos* has a conserved role as a posterior patterning center component in *Nasonia*, and represses the translation of *Nv-hb* mRNA, similar to its *Drosophila* counterpart [17]. In addition, it was shown that the function of *Nv-nos* is dependent on *Nasonia oskar*, showing that the connection between *nanos* and the germ plasm was likely present in the common ancestor of holometabolous insects [18].

Lepidoptera

Although the Lepidoptera are spectacularly diverse, they are relatively understudied when it comes to early AP patterning. Recently, studies in the silkmoth *Bombyx mori* (*Bm*) have begun to overturn this oversight. Like *Nasonia*, *Bm-otd* is localized to the anterior pole of the oocyte and embryo, and is required for patterning all of the head segments [19•]. The anterior patterning function of *Bm-otd* is balanced by a strong influence of *Bm-cad* from the posterior. The way these patterning centers interact to specify the *Bm-Kruppel (Bm-Kr)* expression domain is of high interest. In the absence of *Bm-otd*, *Bm-Kr* is unaffected, while knockdown of *Bm-cad* severely reduces *Bm-Kr* expression. When both of these genes are knocked down together, *Bm-Kr* reappears strongly, in an anteriorly expanded domain, indicating that *Bm-otd* primarily serves to repress *Bm-Kr*, and that another factor in addition to *Bm-Cad* can activate *Bm-Kr* [19•].

Results regarding a Nanos-based posterior center are similarly complicated. In *Bombyx* there are 4 *nanos* orthologs, none of which are localized to the posterior pole during oogenesis, making it unlikely that any of them play a role in establishing the initial polarity of the embryo [20]. Interestingly, one *Bm-nos* ortholog (*Bm-nosP*) is expressed zygotically in a broad posterior domain, and is upregulated after *Bm-cad* pRNAi, but is unaffected in *Bm-otd* RNAi. It was proposed that this regulatory milieu may help to stabilize AP patterning by acting as a buffer between the anterior and posterior patterning centers [19•].

Coleoptera

The first published account of a morphogen role for *otd* was in the beetle *Tribolium*, where it was reported that *Tc-otd1* had a broad anterior patterning role and that it acted in cooperation with *Tc-hb* [11]. Embryonic patterning in *Tribolium*, is of great interest because it undergoes 'short germ' development, where in general the portion of the egg surface that will give rise to the embryonic primordium is small, where only a few segments are patterned before gastrulation, and where the remaining patterning occurs progressively in an extending 'germ band' [21]. Classical experiments indicated that short germ embryos would likely rely more strongly on posterior centers for patterning, partially because the embryonic rudiment generally forms near to the posterior pole, at a significant distance from the anterior [22].

The generation of the presumed Tc-Otd and Tc-Hb protein gradients appeared to fit this posterior center model. Both *Tc-otd* and *Tc-hb* mRNA are provided ubiquitously maternally, while their proteins appeared to retract from the posterior, forming transient protein gradients [11]. Both *Tc-otd1* and *Tc-hb* mRNAs have Nanos response elements (NREs) in their 3′ UTRs, which would indicate that *Tc-nos* could be localized at the posterior, and serve as the posterior center and main source of AP polarity in the embryo [12].

This simple model was later exploded by further examination of the phenotypes and expression patterns of the major players. First it was shown that the presumed role in blastodermal fate specification was in fact a combination of non-specific disruption of extending germband