

Mini-Review

An ancestral regulatory network for posterior development in arthropods

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Key words: spider, arthropod, Wnt signaling, Delta-Notch signaling, caudal, segmentation, growth zone

A number of recent studies have investigated posterior development in several different arthropods. As previously found in spiders, it has been discovered that Delta-Notch signaling is required for the development of posterior segments in an insect, the cockroach *Periplaneta americana*. Furthermore analysis of *Wnt8* function in the spider *Achaearanea tepidariorum* and the beetle *Tribolium castaneum* demonstrates that this Wnt ligand is required for the establishment of the growth zone and development of posterior segments in both these arthropods. Taken together these studies provide an interesting insight into the architecture of the genetic network that regulated posterior development in the common ancestor of the arthropods.

Introduction

During embryogenesis in the fruit fly, *Drosophila melanogaster*, all segments are specified almost simultaneously. This is called the long germ mode of development. In contrast, most other arthropods exhibit short germ development, during which posterior segments are added sequentially from a posteriorly located growth zone.¹ Short germ development appears to be an ancestral feature of arthropods.² Indeed it appears that the genetic regulation underlying posterior development in *Drosophila* is highly derived.³ All animals are of course a composite of both retained ancestral traits and derived features, therefore comparisons of different short germ arthropods has the potential to reveal the ancestral regulatory network for posterior development in these animals.

A number of recent studies by Bolognesi and colleagues, Pueyo and colleagues and us have investigated posterior development in two insects, the red flour beetle *Tribolium castaneum* and the cockroach *Periplaneta americana*, and a chelicerate, the common house spider *Achaearanea tepidariorum*.⁴⁻⁶ From these and many informative previous studies a picture is now emerging of some of the genes and pathways that may have been responsible for the regulation of posterior development in the common ancestor of arthropods.

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Submitted: 12/19/08; Accepted: 12/23/08

Previously published online as a *Communicative & Integrative Biology* E-publication: <http://www.landesbioscience.com/journals/cib/article/7710>

Delta-Notch Signaling in Posterior Segmentation

Although the Delta-Notch signaling pathway is required for segmentation in vertebrates, this pathway is not involved in segmentation in *Drosophila*.^{1,3} However, functional analysis in the spider *Cupiennius salei* surprisingly revealed that Delta-Notch signaling is required for generating the posterior segments in this chelicerate.⁷ Knockdown of *Delta* (*Dl*), *Notch* (*N*) and other components of this pathway using embryonic-RNAi in *Cupiennius* disrupted expression of *hairy* (*h*) and manifested as malformed opisthosomal segments.^{7,8} In addition, parental-RNAi (pRNAi) knockdown of Delta-Notch components in a different spider *Achaearanea tepidariorum*, also resulted in truncated embryos, missing all opisthosomal segments, which shows that this pathway is also involved in the early steps of growth zone formation as well as segmentation.⁹

This work in chelicerates naturally led to the question of whether Delta-Notch signaling is also involved in posterior development in other arthropods. The expression of Delta-Notch components in the myriapod *Strigamia maritima* (a centipede) and the insect *Tribolium* is consistent with a role in posterior development and segmentation, but there is as yet no functional evidence for a role of Delta-Notch in this process in these arthropods.^{10,11}

However a recent study of the cockroach *Periplaneta americana* has provided the first functional evidence for the involvement of Delta-Notch signaling in posterior development and segmentation in an insect.⁶ Pueyo and colleagues showed that Delta-Notch signaling is required for the establishment of the growth zone and for the subsequent sequential generation of segments from this tissue. Manipulation of this pathway gave rise to truncated embryos missing all abdominal segments in the most severe cases, which is consistent with a disrupted growth zone. In less severe phenotypes, embryos exhibited some abdominal tissue, but *hedgehog* (*hh*) stripes formed abnormally or were missing, indeed they were able to show that the expression of *hh* in nascent segments required dynamic expression of *Dl* and *h*.⁶

Together the data from spiders and the cockroach suggests that the common ancestor of arthropods employed Delta-Notch signaling for the development of a posterior growth zone and generation of posterior segments arising from this tissue. In contrast, *Drosophila* does not add segments sequentially from a posterior growth zone and this may explain why Delta-Notch signaling is not involved in segmentation in this long germ insect.

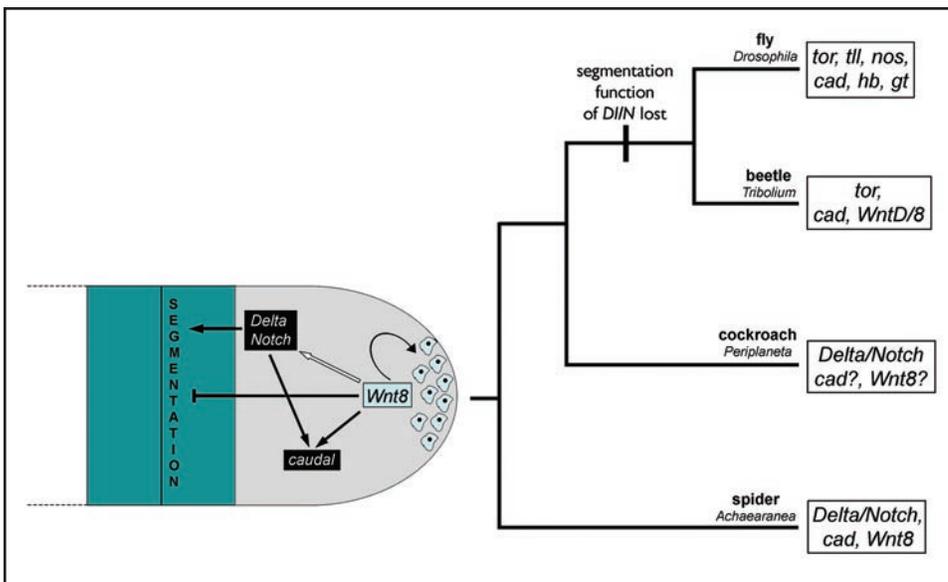


Figure 1. Model of the ancestral regulatory network for posterior development in arthropods and its evolution in insects and spiders. The left-hand illustration, at the base of the tree, shows the possible regulatory network of *Wnt8*, *Delta-Notch* (*DI/N*) and *caudal* (*cad*) in a schematic, generalized growth zone of the hypothetical ancestral arthropod. The last two segments (blue highlighted area) and the unsegmented growth zone (grey highlighted area) are shown with the posterior to the right. Dashed lines indicate more anterior segments. *Wnt8* is thought to establish and maintain a pool of undifferentiated cells at the very posterior of the growth zone (curved arrow pointing to the cells in blue) and may block segmentation (blunt arrow). At the same time, *Wnt8* may be responsible for the dynamic expression of *Delta* and *Notch* because it initiates the clearance of expression of these genes from the very posterior of the growth zone (white arrow), which is needed for the correct induction of the segmentation process. *Wnt8* and the *Delta-Notch* signaling may also be directly or indirectly responsible for the regulation of *cad* (black arrows). While *Delta* and *Notch* play an important role in the segmentation mechanism in spiders and insects like the cockroach, the function of these genes in segmentation may have been lost in the lineage leading to other insects like flies and possibly beetles (indicated by the vertical bar at the branch to beetles and flies).

Wnt Signaling in Posterior Development

It was previously shown that Wnt signaling is involved in posterior development in short germ insects. Knockdown of *armadillo* (*arm*) and *pangolin* in the cricket *Gryllus* and the milkweed bug *Oncopeltus* respectively resulted in truncated embryos with missing abdominal segments.^{12,13} Again, although in *Drosophila* Wnt signaling is not required for the sequential addition of posterior segments, *wg* has a segment polarity function and *WntD/8* is involved in dorso-ventral patterning.^{14,15}

Recent detailed analysis of the function of Wnt signaling in *Tribolium* has now given further insights into the role of Wnt signaling in posterior development in insects.⁴ Bolognesi and colleagues tested the function of the four Wnt ligands expressed in the growth zone in *Tribolium*, *Wnt1/wg*, *Wnt5*, *WntD/8* and *WntA*.^{4,16} They found that loss of *wg* resulted in shorter embryos with smaller segments. In contrast *WntD/8* knockdown resulted in embryos with a smaller growth zone and narrow malformed abdominal segments, or in extreme cases truncated embryos with the growth zone and abdomen completely missing. Interestingly, the extreme *WntD/8* phenotypes were more frequent in *WntD/8* and *wg* double knockdown embryos, suggesting there is some functional redundancy of Wnt ligands in the growth zone. *WntA* and *Wnt5* RNAi knockdown in *Tribolium* had no obvious effect on posterior development.

In contrast to insects relatively little was known about the role of individual Wnt ligands in posterior development in other arthropods. Therefore, we recently investigated the role of *Wnt8* in the spider *Achaearanea*.⁵ Knockdown of *Wnt8* using pRNAi in this spider resulted in posterior defects similar to those when *WntD/8* is knocked down in *Tribolium*. *Achaearanea* *Wnt8* pRNAi phenotypes ranged from embryos with smaller opisthosomal segments to truncated embryos missing all their opisthosomal segments.⁵ Our results suggest *Wnt8* is required for establishing and maintaining the growth zone during opisthosomal development through the maintenance of an undifferentiated pool of cells. Furthermore, our analysis indicates that the mechanism of this function of *Wnt8* is through the direct or indirect regulation of dynamic expression of *DI* in the growth zone, which is required for the correct expression of *caudal* (*cad*) and *twist*. Removal of *Wnt8* appears to arrest dynamic *DI* expression and thus gives rise to extensive ectopic *DI* expression throughout the posterior. This results in premature differentiation of growth zone cells and therefore truncated embryos. Intriguingly a similar role has been suggested for *Wnt8* during somitogenesis in vertebrates.¹⁷

Knock down of *Wnt8* in *Achaearanea* also resulted in fusion of limb buds along the dorso-ventral (D-V) axis suggesting that *Wnt8* is also involved in patterning the spider embryo along the D-V axis. A role in D-V patterning has also been described for *Wnt8* in vertebrates.⁵ Intriguingly, although *Drosophila* does not require *Wnt8* for posterior development this insect may have retained an ancestral role of *Wnt8* along the D-V axis.¹⁵

These analyses of the function of *Wnt8* in a beetle and spider add to previous studies of Wnt signaling in insects and show that not only was Wnt signaling integral to the regulation of posterior development in the ancestral arthropod, but that the *Wnt8* and *Wnt1/wg* ligands likely played key roles. Additional analysis of these and other Wnt signaling components in spiders, beetles and other arthropods should prove rewarding in further dissecting the role of this pathway in posterior development.

Caudal and Posterior Development

It is clear that *cad/cdx* genes are key factors in the posterior development of many animals suggesting that these genes had an ancient role in this process.^{18,19} In spiders it appears that *cad* is expressed zygotically throughout the addition of posterior segments from the growth zone,^{5,20} but its expression is not consistent with a role in anterior patterning as found in other arthropods.^{19,21} Experiments in spiders have revealed that the *Delta-Notch* and *Wnt8* signaling pathways are both involved in the regulation of *cad* expression

in the posterior, although whether this is direct or indirect is not known. There is evidence that *cad* expression is also regulated by Wnt signaling in short germband insects like the cricket and the beetle. Knockdown of *arm* in *Gryllus* results in a loss of *cad* expression, although the specific Wnt ligand involved has not yet been determined, and although *cad* expression in *Tribolium* is regulated by the *torso* pathway, this may be mediated by Wnt signaling.^{21,22} Therefore it is possible that Wnt signaling and perhaps Delta-Notch played an important role in the regulation of zygotic *cad* expression in the arthropod common ancestor.

An Ancestral Regulatory Network for Posterior Development?

Although lineage specific differences have evolved, comparative studies using a growing number of arthropods are beginning to reveal a common core of factors and pathways involved in the regulation of posterior development in these animals (Fig. 1). This suggests that the common ancestor of arthropods employed the Delta-Notch pathway, Wnt signaling possibly through *Wnt8*, and *cad* for the establishment of a posterior growth zone and the sequential generation of segments from this structure (Fig. 1). A picture of how these factors interact is also beginning to emerge, for example it appears that Wnt signaling is required for zygotic *cad* expression in different arthropods (Fig. 1). However, much work remains to be done in dissecting the precise regulatory interactions between these factors and other components involved in posterior development.

It is striking that these three core factors in posterior development in arthropods are also involved in posterior development in vertebrates.^{18,23-25} This suggests that this regulatory network was present in the common ancestor of arthropods and vertebrates. However, to address the question of whether the common ancestor of bilaterally symmetrical animals, Urbilateria,²⁶ used this network for the generation of segments as well as posterior development requires further experimental efforts in vertebrates and arthropods, and functional studies in other animal phyla like annelids will be particularly illuminating with respect to these questions.

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