Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

*Curr Opin Insect Sci*. Author manuscript; available in PMC 2023 April 01.

Published in final edited form as:

*Curr Opin Insect Sci*. 2022 April ; 50: 100877. doi:10.1016/j.cois.2022.100877.

# Evolution and loss of ß-catenin and TCF-dependent axis specification in insects

**Urs Schmidt-Ott**[1], **Yoseop Yoon**[2]

[1)]University of Chicago, Dept. of Organismal Biology and Anatomy, 1027 East 57th Street, Chicago, IL 60637, USA

[2)]University of California, Irvine, Dept. of Microbiology and Molecular Genetics, School of Medicine, 811 Health Sciences Rd, Med Sci B262, CA 92617, USA

## Summary

Mechanisms and evolution of primary axis specification in insects are discussed in the context of the roles of ß-catenin and TCF in polarizing metazoan embryos. Three hypotheses are presented. First, insects with sequential segmentation and posterior growth use cell-autonomous mechanisms for establishing embryo polarity via the nuclear ratio of ß-catenin and TCF. Second, TCF homologs establish competence for anterior specification. Third, the evolution of simultaneous segmentation mechanisms, also known as long-germ development, resulted in primary axis specification mechanisms that are independent of ß-catenin but reliant on TCF, a condition that preceded the frequent replacement of anterior determinants in long germ insects.

## Introduction

Many animals use ß-catenin to polarize embryo development [1, 2]. This molecule is a conserved structural component of cadherin-based adherens junctions, but it also functions as a transcriptional co-factor and regulator of chromatin structure [3, 4]. Accumulation of ß-catenin in the nucleus is regulated by a cytoplasmic ß-catenin destruction complex, which can be disrupted by Wnt signaling (canonical Wnt signaling) [5] or independent of Wnt ligands through various lineage-specific mechanisms [6–10]. Disassembly of the destruction complex causes nuclear ß-catenin levels to rise and can induce the formation of ectopic axial organizers in organisms as diverse as juvenile sponges [11, 12], cnidarians [13–16], sea urchins [9, 10, 17], and vertebrates [6]. It is also required for establishing anterior-posterior polarity in regenerating bilaterians [18–21]. Therefore, ß-catenin-dependent primary axis specification is likely a shared heritage of the Metazoa. While in general peak levels of nuclear ß-catenin are required for specifying the posterior pole of the embryo and antagonistic effects are associated with the anterior pole [1], many lineage-specific innovations in embryogenesis have obscured this common heritage and thus the comparison of metazoan body plans (Box 1).

This manuscript version is made available under the CC-BY-NC-ND 4.0 license https://creativecommons.org/licenses/by-nc-nd/4.0/

uschmid@uchicago.edu .

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Nuclear ß-catenin breaks symmetry in axis specification and in binary cell fate decisions by binding transcription factors of the T-cell factor (TCF)/ Lymphoid enhancer-binding factor (Lef) protein family (henceforth TCF) [2, 3]. The nematode *Caenorhabditis elegans* (*C. elegans*) has emerged as an important model to study this mechanism in the context of reiterated ß-catenin-dependent binary cell fate specifications, which occur throughout development [7, 8, 22–24]. We will use this model to explain how ß-catenin and TCF break symmetry before reviewing the evolution of these factors in primary axis specification mechanisms in the insect lineage. The binary cell fate specifications in *C. elegans* are driven by functionally distinct ß-catenin paralogs, called SYS-1 and WRM-1. SYS-1-bound POP-1 (TCF) enables the expression of POP-1 target genes, whereas the ß-catenin related protein WRM-1, which is not conserved in other species, promotes phosphorylation and nuclear export of POP-1. SYS-1 and WRM-1 are regulated downstream of the Wnt receptor complex and Dishevelled, a regulator of the ß-catenin destruction complex. Exposure to Wnt signal orients the spindle of the dividing cell. As a result, SYS-1 and WRM-1 preferentially accumulate in the nuclei of the posterior-born daughter cells and the two daughter cells exhibit opposite bias of POP-1 and SYS-1. However, Wnt ligands are not an obligatory component of this cell fate specification mechanism and some cells of the *C. elegans* embryo undergo ß-catenin-dependent binary cell fate specifications independent of Wnt.

The principle of breaking symmetry via the nuclear ratio of ß-catenin and TCF seems to be applicable to ß-catenin-dependent axis specification mechanisms in a broad range of metazoans. Below, we examine how this mechanism evolved in arthropods – insects in particular, which in turn may inform our understanding of the role of TCF in primary axis specification mechanisms in other metazoans. To meet editorial constraints on the length of this short opinion piece, we have prioritized recent primary research articles and refer the reader to references cited therein for a more complete coverage of the vast literature on axis specification in insects and other metazoans.

## TCF and ß-catenin requirements in early arthropod embryos with sequential segmentation

In early embryos of arthropods, the regulation of TCF (Pangolin) activity via ß-catenin (Armadillo) has been implicated in establishing segment boundaries [25, 26], posterior specification and growth [27–30], and embryo polarity [31–33]. Of these, only a role in establishing segmental boundaries is conserved in the genetic model organism *Drosophila melanogaster* [5, 25]. The role of TCF/Pangolin and ß-catenin/Armadillo in specifying the posterior embryo and in promoting growth has been documented (or inferred) in insects and spiders with sequential segmentation [27–30]. This developmental process requires zygotic signaling through Wnt ligands. The Wnt-activated receptor complex of Frizzled and Arrow (LRP5/6) sequesters Axin at the cell membrane and, through a process that also involves Dishevelled, triggers disintegration or inactivation of the ß-catenin destruction complex and increases nuclear levels of ß-catenin[5].

A maternal role of the nuclear ratio of ß-catenin/Armadillo and TCF/Pangolin in establishing embryo polarity has been proposed based on experiments in the beetle *Tribolium castaneum*

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[31–33], a species with posterior growth zone and sequential segment addition under the control of zygotic Wnt signaling. In Tribolium, maternal transcripts of *axin* are localized at the anterior pole of the egg and essential for anterior specification and embryo polarity [32–34], presumably because the maternal Axin gradient reduces anterior ß-catenin levels. Additionally, maternal *Tcf/pangolin* transcript is enriched at the anterior pole of the Tribolium egg [34]. The reason for this is less clear, given that *Tcf/pangolin* knockdown blocks posterior specification, growth, and segmentation [27, 32]. However, maternal TCF/Pangolin seems to aid in suppressing posterior cell fate specification in the anterior embryo [35], presumably by reinforcing a low nuclear ß-catenin:TCF ratio at the anterior pole of the egg.

## TCF-related anterior determinants in insects with simultaneous segmentation

In species with simultaneous segmentation (long-germ development), such as the dipterans (true flies), maternal *axin* mRNA does not seem to be localized in the anterior egg. However, some of these species use TCF/Pangolin as anterior determinant (Figure 1, Table 1) [36]. For example, in anopheline mosquitoes, a maternal transcript isoform of TCF/Pangolin is localized at the anterior pole of the egg and required for anterior specification. Knockdown of *Tcf/pangolin* in early Anopheles embryos by RNAi prevents anterior specification and can cause a mirror image duplication of the posterior end in addition to segmentation defects that probably reflect perturbed zygotic roles of ß-catenin-bound TCF/Pangolin. Crane flies, which represent a different lineage of lower dipterans, also accumulate maternal *Tcf/pangolin* transcript in the anterior egg. Of particular interest is the case of Chironomus midges, which are related to mosquitoes. In Chironomus, *Tcf/pangolin* is conserved but only expressed zygotically while a specialized *Tcf/pangolin* paralog, called *panish*, assumes a strictly maternal role as anterior determinant [37]. This apparent case of gene duplication with subfunctionalization of maternal and zygotic *Tcf/pangolin* roles provides an opportunity to assess the maternal function of TCF/Pangolin in primary axis specification of a long-germ insect without constraints imposed by TCF/Pangolin functions in posterior growth and segmentation. The zygotic TCF/Pangolin functions in posterior growth and segmentation require interaction with ß-catenin via the N-terminal ß-catenin-binding domain and DNA-binding via High Mobility Group (HMG) domain, as well as a cysteine-clamp (C-clamp) [3, 38]. The HMG domain, which bends DNA at its target sites, is considered essential for the function of TCF homologs, whereas the C-clamp is thought to provide added target gene specificity via a GC-rich motif near HMG binding sites. However, Panish lacks the ß-catenin-binding domain and the HMG domain, and only retains the C-clamp. Taken together, the findings in anopheline mosquitoes, crane flies, and Chironomus suggest that ß-catenin may have become dispensable for specifying embryo polarity in long germ insects. Moreover, conservation of the C-clamp and loss of the HMG domain in Panish suggest that the C-clamp plays a critical role in the function of anterior determinants of the TCF/Pangolin protein family. What this function might be will be discussed in the context of novel TCF-unrelated anterior determinants that evolved in other dipteran insects.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

## TCF-unrelated anterior determinants in dipteran insects

Long-germ insects have evolved many mechanisms for establishing embryo polarity that are based on maternal mRNA localizations at the anterior pole or both the anterior and posterior poles of the egg [36, 37, 39–43]. These evolutionary innovations may have been unleashed after removing the constraint of maintaining a segment addition zone under the control of posteriorizing ß-catenin signal and making embryo polarity dependent on anterior TCF/Pangolin activity. They seem to have occurred independently in many lineages and often led to a replacement of TCF/Pangolin as symmetry-breaking anterior determinant. For example, among the lower dipterans (Nematocera), moth flies and culicine mosquitoes evolved new anterior determinants that are encoded by unrelated C2H2 zinc-finger genes, including *zic*/*odd-paired* and *cucoid*, respectively [36], while among the higher dipterans (Brachycera), cyclorrhaphan flies evolved the anterior determinant gene *bicoid* from a duplicated Hox3 ortholog [44, 45]. Dipterans may have evolved many more anterior determinants, given that *bicoid* was repeatedly lost during the radiation of cyclorrhaphan flies [37].

The diversity of anterior determinants in dipteran insects raises the question of whether they share a common molecular mechanism for breaking symmetry in the context of zygotic genome activation. In Drosophila, zygotic genome activation is largely driven by a small number of ubiquitous pioneer factors that initiate chromatin accessibility at thousands of cis-regulatory elements [46–49]. The anterior-to-posterior Bicoid gradient polarizes this process along the primary axis by driving chromatin accessibility at cis-regulatory elements of target genes that need to be activated for anterior specification [50–52]. Optogenetic experiments indicate that robust pattern formation continuously requires Bicoid from the time shortly after blastoderm formation onwards (i.e., after nuclear cycle 10) until the onset of gastrulation[53]. This phase of development corresponds to the blastula stage during which other organisms specify embryo polarity through TCF and ß-catenin [6, 10, 17, 54, 55]. The same study also shows that the prospective head region, where Bicoid builds up first, is most sensitive to shortened Bicoid exposure[56]. Therefore, timing of naïve chromatin exposure to Bicoid, rather than the formation of a static spatial concentration gradient of Bicoid, may enable its anterior determinant function.

Whether the anterior determinants of other dipterans function in a similar manner is currently unknown but a possibility. Drosophila Odd-paired is required for chromatin accessibility at specific target genes during gastrulation [52, 57], and may have been “preadapted” for breaking symmetry at the chromatin level during zygotic genome activation as anterior determinant in moth flies. The function of Cucoid in mosquitoes remains poorly known; while *cucoid* is a conserved single copy gene across lower dipterans, its evolution in higher dipterans is complex and not well understood. TCF and other HMG proteins, have been found to exhibit high affinity to nucleosomes [58–60] and have been described as a platform for broad chromatin remodeling in response to ß-catenin binding and other factors [3]. However, the TCF-related anterior determinant of Chironomus, Panish, has lost the HMG-domain, and whether its C-Clamp can engage with nucleosome-bound DNA is unknown but should not be ruled out. Panish seems to establish competence for anterior development throughout the Chironomus blastoderm. We infer this from the Panish-dependent RNAi phenotype of *tailless*, which is likely a direct target gene [37]. In

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Chironomus, *tailless* transcription is initiated zygotically in a posterior-to-anterior gradient and suppresses head formation in the posterior embryo, thereby preventing the formation of embryos that only consist of mirror-image double heads. In the anterior embryo, Panish represses *tailless* and prevents embryos from developing as mirror image double abdomens. However, even though *tailless* functions downstream of Panish, double knockdown of *panish* and *tailless* resulted in double abdomens, indicating that in the absence of Panish the posterior blastoderm loses its competence for head formation. Thus, it is conceivable that Panish primes naive blastoderm cells throughout the embryo for anterior specification while early zygotic *tailless* expression – a negative target of Panish in the anterior embryo - limits its realization.

## Evolution of new anterior determinants in dipteran insects

It is by now well established that anterior determinants frequently changed during the dipteran radiation. Moreover, at least some dipterans abandoned ß-catenin-dependent or ß-catenin and TCF-dependent primary axis specification mechanisms (e.g., Drosophila). If this trend was enabled by the evolution of simultaneous segmentation mechanisms and the loss of ß-catenin-dependent posterior growth zone specification, the independent evolution of long-germ development in other insects, including beetles and wasps, likewise may have resulted in many new lineage-specific anterior determinants.

How did new anterior determinants adopt their fundamental role in development? One approach to this question in recent years has been to reconstruct ancestral amino acid sequences of the DNA-binding homeodomains of Bicoid (AncBcd HD) and its immediate Hox3/Zerknullt precursor (AncZB HD) and to compare the function of diagnostic residues that distinguish these proteins using *in vitro* DNA binding assays and rescue experiments in Drosophila [45, 61]. The *in vitro* assays (electro mobility shift assays, protein binding microarrays) quantitatively confirmed that DNA motif recognition differences between AncBcd and AncZB HDs mostly depend on a single amino acid replacement (Q50K) in the recognition helix of the Bicoid homeodomain, but full functionality of AncBcd HD in Drosophila embryos was found to additionally depend on other diagnostic residues. These findings seem to suggest that Bicoid acquired its function gradually. However, it is unknown how any of the Bicoid mutations affected the ancestral organism and whether they occurred before or after Bicoid assumed the role of anterior determinant.

Another approach was to investigate phylogenetically old genes, such as *odd-paired*, *cucoid*, and *TCF/pangolin*, that acquired this function by localizing a maternal transcript isoform with distinct 5’ end (via alternative transcription) or 3’ end (via alternative polyadenylation) at the anterior pole of the egg[36]. For technical reasons, these studies were mostly carried out in the moth fly *Clogmia albipunctata*. In this species, maternal and zygotic *odd-paired* transcripts with distinct 5’ ends serve non-overlapping roles in anterior specification and segmentation, respectively. It was found that maternal and zygotic *odd-paired* sequences as well as *odd-paired* homologs from other species, such as Drosophila, can serve as anterior determinant in Clogmia when provided ectopically in the posterior embryo at a very early stage of development. These observations indicate that Clogmia *odd-paired* evolved the anterior determinant function without essential changes to the Odd-paired protein via

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

co-option of localized maternal *odd-paired* activity. Given that the anterior determinant functions of *cucoid* in culicine mosquitoes and of Tcf/pangolin in anopheline mosquitoes also rest on localized alternative transcript isoforms, co-option of localized (or locally active) transcript isoforms might be a common mechanism by which preexisting genes adopt an additional function as anterior determinant. It is therefore possible that *panish* and *bicoid* inherited their specific anterior determinant function from multifunctional progenitor genes by gene duplication and subfunctionalization. In this scenario, which has been discussed elsewhere in more detail [36], the Panish and Bicoid proteins retained their inherited anterior determinant function despite divergence from their evolutionary sisters (TCF/Pangolin, Hox3/Zerknullt) through repeated cycles of compensatory evolution [36, 45]. TCF/Pangolin and Hox3/Zerknullt cannot substitute for the anterior determinant functions of Panish and Bicoid in rescue experiments. Therefore, it is possible that Panish and Bicoid benefit their organisms by capping pleiotropy of their multifunctional ancestral progenitors and reducing the risk of genetic mis-regulation. However, the frequent replacement of anterior determinants suggests that other factors also drive or enable their rapid evolution.

## Conclusions and outlook

Our understanding of primary axis specification mechanisms in insects remains surprisingly incomplete (Figure 1). Three hypotheses are proposed in the hope that they may help to guide future research.

1. *Short-germ insects use cell-autonomous (Wnt-independent) mechanisms to establish embryo polarity via the nuclear ratio of ß-catenin and TCF:* Asymmetry in the nuclear ratio of ß-catenin and TCF is widely used to establish embryo polarity in metazoan development. This ratio is regulated via cytoplasmic ß-catenin destruction and the local enrichment or depletion of TCF. Canonical Wnt signaling interferes with cytoplasmic ß-catenin degradation and thereby increases nuclear ß-catenin levels. However, the importance of Wnt ligands in establishing embryo polarity under normal conditions may have been overstated. In frogs and fish, targeted Axin degradation establishes the axial organizer via nuclear ß-catenin accumulation. This process is regulated by the localization of maternal *huluwa* mRNA and is independent of Wnt ligands [6]. In invertebrates, the regulation of nuclear ß-catenin levels prior to gastrulation is less well understood but compelling evidence for a role of Wnts in establishing embryo polarity or early blastomere specification is rare (e.g., *C. elegans* [62]). In cnidarians and ctenophores, axis specification occurs during the first cleavage cycle [63] and seems to be regulated cell-autonomously via polarized Dishevelled protein accumulation [64, 65]. In sea urchins, high levels of maternal Axin suppress nuclear ß-catenin accumulation and posterior cell fate specification in anterior blastomeres cell-autonomously [10]. Unlike in frogs and fish, the upstream regulator of posterior Axin degradation in sea urchins remains unknown. In short-germ insects (Tribolium), too, high levels of maternal Axin suppress posterior cell fate specification in the anterior embryo [33]. However, in this species, maternal mRNA localization at the anterior egg pole, rather than localized Axin degradation or Axin sequestration in response to posterior

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Wnt signaling, establishes embryo polarity, apparently in parallel with other much weaker polarizing mechanisms that remain less well understood [31]. Knockdown of the Wnt receptor complex or Wnt ligands disrupts the segment addition zone and segmentation, but it does not abolish embryo polarity in Tribolium [27, 31–33, 66]. While residual Wnt receptor activity has not been ruled out in these knockdown experiments, available evidence does not suggest an essential role of Wnt ligands in establishing embryo polarity in insects.

2. *TCF/Pangolin establishes competence for anterior specification:* Short-germ insects use ß-catenin-bound TCF/Pangolin to establish and maintain the segment addition zone (growth zone), rather than embryo polarity *per se*. However, in Tribolium, the only arthropod with sequential segmentation in which TCF/Pangolin function has been studied in the context of primary axis specification, anterior specification seems to require depletion of nuclear ß-catenin (in response to high Axin levels). This finding suggests a critical role of TCF/Pangolin in this process, but knockdown of *Tcf/pangolin* had only mild effects on anterior specification [35]. The competence of these embryos to form anterior structures could be due to residual TCF/Pangolin activity and/or additional polarity-generating genetic mechanisms, such as asymmetric anterior Torso signaling through dpERK [31]. A knockout of maternal Tcf/Pangolin activity would be required to distinguish between these hypotheses but whether such embryos can be obtained is unclear. While the role of maternal *Tcf/pangolin* in early Tribolium embryos remains poorly understood, Panish seems necessary to establish competence for anterior specification throughout the early Chironomus embryo, i.e., even in the posterior blastoderm where its expression level is low. Panish may have inherited this function from TCF/Pangolin because some other lower dipterans use TCF/Pangolin as anterior determinant (Figure 1, Table 1). It is therefore conceivable that the establishment of anterior competence through TCF/Pangolin reflects an ancestral function of this protein in axis specification.

3. *Loss of ß-catenin as axis determinant in long-germ insects preceded evolutionary plasticity of anterior determinants:* The phylogenetic occurrence of anterior determinants in dipterans suggests that axis specification through localized TCF/Pangolin preceded the frequent replacement of anterior determinants during the dipteran radiation. Moreover, given the lack of evidence for asymmetric maternal ß-catenin regulation in these species, asymmetric TCF/Pangolin activity may have evolved to compensate for the loss of asymmetric ß-catenin activity. Whether this condition is evolutionarily unstable and led to the evolution of many alternative anterior determinants, including Bicoid, is currently unknown. A better understanding of the molecular mechanisms by which new anterior determinants specify embryo polarity in different species should help to illuminate this question.

## Acknowledgements

During the preparation of this manuscript Kenneth Veland Halberg (University of Copenhagen) and Jeremy Lynch (University of Illinois at Chicago) kindly shared unpublished maternal transcriptome data for Tribolium. Shelby

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Blythe (Northwestern University) and Christopher Lowe (Stanford University) and Ezzat El-Sherif (University of Erlangen) shared valuable insights. YY was the recipient of an award of University of Chicago Henry Hinds Funds for Graduate Student Research in Evolutionary Biology. Research support was provided by the National Institute of Health (R01GM127366).

## References

1. Petersen CP, and Reddien PW (2009). Wnt signaling and the polarity of the primary body axis. Cell 139, 1056–1068. [PubMed: 20005801]

2. Loh KM, van Amerongen R, and Nusse R (2016). Generating Cellular Diversity and Spatial Form: Wnt Signaling and the Evolution of Multicellular Animals. Dev Cell 38, 643–655. [PubMed: 27676437]

3. Valenta T, Hausmann G, and Basler K (2012). The many faces and functions of β-catenin. Embo j 31, 2714–2736. [PubMed: 22617422]

4. Blythe SA, Cha SW, Tadjuidje E, Heasman J, and Klein PS (2010). beta-Catenin primes organizer gene expression by recruiting a histone H3 arginine 8 methyltransferase, Prmt2. Dev Cell 19, 220–231. [PubMed: 20708585]

5. Wiese KE, Nusse R, and van Amerongen R (2018). Wnt signalling: conquering complexity. Development 145.

••6. Yan L, Chen J, Zhu X, Sun J, Wu X, Shen W, Zhang W, Tao Q, and Meng A (2018). Maternal Huluwa dictates the embryonic body axis through beta-catenin in vertebrates. Science 362.This study identifies a Wnt-independent mechanism for ß-catenin-dependent axis specification in Xenopus and zebrafish.

7. Park FD, and Priess JR (2003). Establishment of POP-1 asymmetry in early C. elegans embryos. Development 130, 3547–3556. [PubMed: 12810601]

••8. Zacharias AL, Walton T, Preston E, and Murray JI (2015). Quantitative Differences in Nuclear beta-catenin and TCF Pattern Embryonic Cells in C. elegans. PLoS Genet 11, e1005585. [PubMed: 26488501] This study provides a quantitative framework for interpreting the roles of ß-catenin and TCF during binary cell fate specifications throughout most of embryonic development in *C. elegans*.

9. Logan CY, Miller JR, Ferkowicz MJ, and McClay DR (1999). Nuclear beta-catenin is required to specify vegetal cell fates in the sea urchin embryo. Development 126, 345–357. [PubMed: 9847248]

•10. Sun H, Peng CJ, Wang L, Feng H, and Wikramanayake AH (2021). An early global role for Axin is required for correct patterning of the anterior-posterior axis in the sea urchin embryo. Development 148.This study provides evidence for cell-autonomous regulation of ß-catenin and embryo polarity in a sea urchin model.

11. Windsor PJ, and Leys SP (2010). Wnt signaling and induction in the sponge aquiferous system: evidence for an ancient origin of the organizer. Evol Dev 12, 484–493. [PubMed: 20883217]

12. Adamska M, Degnan SM, Green KM, Adamski M, Craigie A, Larroux C, and Degnan BM (2007). Wnt and TGF-beta expression in the sponge Amphimedon queenslandica and the origin of metazoan embryonic patterning. PLoS One 2, e1031. [PubMed: 17925879]

13. Kraus Y, Aman A, Technau U, and Genikhovich G (2016). Pre-bilaterian origin of the blastoporal axial organizer. Nat Commun 7, 11694. [PubMed: 27229764]

14. Broun M, Gee L, Reinhardt B, and Bode HR (2005). Formation of the head organizer in hydra involves the canonical Wnt pathway. Development 132, 2907–2916. [PubMed: 15930119]

15. Gee L, Hartig J, Law L, Wittlieb J, Khalturin K, Bosch TC, and Bode HR (2010). beta-catenin plays a central role in setting up the head organizer in hydra. Dev Biol 340, 116–124. [PubMed: 20045682]

16. Hobmayer B, Rentzsch F, Kuhn K, Happel CM, von Laue CC, Snyder P, Rothbächer U, and Holstein TW (2000). WNT signalling molecules act in axis formation in the diploblastic metazoan Hydra. Nature 407, 186–189. [PubMed: 11001056]

17. Wikramanayake AH, Hong M, Lee PN, Pang K, Byrum CA, Bince JM, Xu R, and Martindale MQ (2003). An ancient role for nuclear beta-catenin in the evolution of axial polarity and germ layer segregation. Nature 426, 446–450. [PubMed: 14647383]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

18. Gurley KA, Rink JC, and Sanchez Alvarado A (2008). Beta-catenin defines head versus tail identity during planarian regeneration and homeostasis. Science 319, 323–327. [PubMed: 18063757]

19. Petersen CP, and Reddien PW (2008). Smed-betacatenin-1 is required for anteroposterior blastema polarity in planarian regeneration. Science 319, 327–330. [PubMed: 18063755]

20. Iglesias M, Gomez-Skarmeta JL, Salo E, and Adell T (2008). Silencing of Smed-betacatenin1 generates radial-like hypercephalized planarians. Development 135, 1215–1221. [PubMed: 18287199]

21. Srivastava M, Mazza-Curll KL, van Wolfswinkel JC, and Reddien PW (2014). Whole-body acoel regeneration is controlled by Wnt and Bmp-Admp signaling. Curr Biol 24, 1107–1113. [PubMed: 24768051]

22. Kaletta T, Schnabel H, and Schnabel R (1997). Binary specification of the embryonic lineage in Caenorhabditis elegans. Nature 390, 294–298. [PubMed: 9384382]

23. Lin R, Hill RJ, and Priess JR (1998). POP-1 and anterior-posterior fate decisions in C. elegans embryos. Cell 92, 229–239. [PubMed: 9458047]

24. Huang S, Shetty P, Robertson SM, and Lin R (2007). Binary cell fate specification during C. elegans embryogenesis driven by reiterated reciprocal asymmetry of TCF POP-1 and its coactivator beta-catenin SYS-1. Development 134, 2685–2695. [PubMed: 17567664]

25. Swarup S, and Verheyen EM (2012). Wnt/Wingless signaling in Drosophila. Cold Spring Harb Perspect Biol 4.

••26. Clark E, Peel AD, and Akam M (2019). Arthropod segmentation. Development 146.This review examines the genetic basis of sequential and simultaneous segmentation mechanisms in arthropods.

27. Bolognesi R, Farzana L, Fischer TD, and Brown SJ (2008). Multiple Wnt genes are required for segmentation in the short-germ embryo of Tribolium castaneum. Curr Biol 18, 1624–1629. [PubMed: 18926702]

28. McGregor AP, Pechmann M, Schwager EE, Feitosa NM, Kruck S, Aranda M, and Damen WG (2008). Wnt8 is required for growth-zone establishment and development of opisthosomal segments in a spider. Curr Biol 18, 1619–1623. [PubMed: 18926703]

•29. Setton EVW, and Sharma PP (2021). A conserved role for arrow in posterior axis patterning across Arthropoda. Dev Biol 475, 91–105. [PubMed: 33607111] The study reassesses and expands knowledge on the role of canonical Wnt siganling during anterior-posterior axis specification in hemimetabolous insects and spiders.

30. Chesebro JE, Pueyo JI, and Couso JP (2013). Interplay between a Wnt-dependent organiser and the Notch segmentation clock regulates posterior development in Periplaneta americana. Biol Open 2, 227–237. [PubMed: 23430316]

••31. Ansari S, Troelenberg N, Dao VA, Richter T, Bucher G, and Klingler M (2018). Double abdomen in a short-germ insect: Zygotic control of axis formation revealed in the beetle Tribolium castaneum. Proc Natl Acad Sci U S A 115, 1819–1824. [PubMed: 29432152] This study provides a detailed analysis of the mechanisms underlying embryo polarity in short-germ insect.

32. Fu J, Posnien N, Bolognesi R, Fischer TD, Rayl P, Oberhofer G, Kitzmann P, Brown SJ, and Bucher G (2012). Asymmetrically expressed axin required for anterior development in Tribolium. Proc Natl Acad Sci U S A 109, 7782–7786. [PubMed: 22552230]

•33. Prühs R, Beermann A, and Schröder R (2017). The Roles of the Wnt-Antagonists Axin and Lrp4 during Embryogenesis of the Red Flour Beetle Tribolium castaneum. J Dev Biol 5.This study establishes a critical role of Axin in establishing embryo polarity in Tribolium.

34. Bucher G, Farzana L, Brown SJ, and Klingler M (2005). Anterior localization of maternal mRNAs in a short germ insect lacking bicoid. Evol Dev 7, 142–149. [PubMed: 15733312]

35. El-Sherif E, Zhu X, Fu J, and Brown SJ (2014). Caudal regulates the spatiotemporal dynamics of pair-rule waves in Tribolium. PLoS Genet 10, e1004677. [PubMed: 25329152]

••36. Yoon Y, Klomp J, Martin-Martin I, Criscione F, Calvo E, Ribeiro J, and Schmidt-Ott U (2019). Embryo polarity in moth flies and mosquitoes relies on distinct old genes with localized transcript isoforms. Elife 8.This study reports three new anterior determinants in dipteran insects and illuminates the evolutionary mechanism by which new anterior determinants evolve.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

37. Klomp J, Athy D, Kwan CW, Bloch NI, Sandmann T, Lemke S, and Schmidt-Ott U (2015). Embryo development. A cysteine-clamp gene drives embryo polarity in the midge Chironomus. Science 348, 1040–1042. [PubMed: 25953821]

38. Ravindranath AJ, and Cadigan KM (2016). The Role of the C-Clamp in Wnt-Related Colorectal Cancers. Cancers (Basel) 8.

39. Berleth T, Burri M, Thoma G, Bopp D, Richstein S, Frigerio G, Noll M, and Nusslein-Volhard C (1988). The role of localization of bicoid RNA in organizing the anterior pattern of the Drosophila embryo. EMBO J 7, 1749–1756. [PubMed: 2901954]

40. Wang C, and Lehmann R (1991). Nanos is the localized posterior determinant in Drosophila. Cell 66, 637–647. [PubMed: 1908748]

41. Lynch JA, Brent AE, Leaf DS, Pultz MA, and Desplan C (2006). Localized maternal orthodenticle patterns anterior and posterior in the long germ wasp Nasonia. Nature 439, 728–732. [PubMed: 16467838]

42. Olesnicky EC, Brent AE, Tonnes L, Walker M, Pultz MA, Leaf D, and Desplan C (2006). A caudal mRNA gradient controls posterior development in the wasp Nasonia. Development 133, 3973–3982. [PubMed: 16971471]

43. Brent AE, Yucel G, Small S, and Desplan C (2007). Permissive and instructive anterior patterning rely on mRNA localization in the wasp embryo. Science 315, 1841–1843. [PubMed: 17395827]

44. Stauber M, Prell A, and Schmidt-Ott U (2002). A single Hox3 gene with composite bicoid and zerknullt expression characteristics in non-Cyclorrhaphan flies. Proc Natl Acad Sci U S A 99, 274–279. [PubMed: 11773616]

45. Liu Q, Onal P, Datta RR, Rogers JM, Schmidt-Ott U, Bulyk ML, Small S, and Thornton JW (2018). Ancient mechanisms for the evolution of the bicoid homeodomain's function in fly development. Elife 7.

46. Sun Y, Nien CY, Chen K, Liu HY, Johnston J, Zeitlinger J, and Rushlow C (2015). Zelda overcomes the high intrinsic nucleosome barrier at enhancers during Drosophila zygotic genome activation. Genome Res 25, 1703–1714. [PubMed: 26335633]

47. Schulz KN, Bondra ER, Moshe A, Villalta JE, Lieb JD, Kaplan T, McKay DJ, and Harrison MM (2015). Zelda is differentially required for chromatin accessibility, transcription factor binding, and gene expression in the early Drosophila embryo. Genome Res 25, 1715–1726. [PubMed: 26335634]

48. Duan J, Rieder L, Colonnetta MM, Huang A, McKenney M, Watters S, Deshpande G, Jordan W, Fawzi N, and Larschan E (2021). CLAMP and Zelda function together to promote Drosophila zygotic genome activation. Elife 10.

49. Gaskill MM, Gibson TJ, Larson ED, and Harrison MM (2021). GAF is essential for zygotic genome activation and chromatin accessibility in the early Drosophila embryo. Elife 10.

••50. Hannon CE, Blythe SA, and Wieschaus EF (2017). Concentration dependent chromatin states induced by the bicoid morphogen gradient. Elife 6.This study examines the role of Bicoid in shaping chromatin accessibility at different concentration levels without confounding input from other polarizing genetic mechanisms and establishes a role of Bicoid as a pioneer-like factor in the anterior embryo.

51. Haines JE, and Eisen MB (2018). Patterns of chromatin accessibility along the anterior-posterior axis in the early Drosophila embryo. PLoS Genet 14, e1007367. [PubMed: 29727464]

••52. Soluri IV, Zumerling LM, Payan Parra OA, Clark EG, and Blythe SA (2020). Zygotic pioneer factor activity of Odd-paired/Zic is necessary for late function of the Drosophila segmentation network. Elife 9.This study uses ATAC- and ChIP-sequencing approaches in Drosophila to establish a role of Odd-paired in driving chromatin accessibility during early gastrulation of the Drosophila embryo to advance the segmentation process.

••53. Huang A, Amourda C, Zhang S, Tolwinski NS, and Saunders TE (2017). Decoding temporal interpretation of the morphogen Bicoid in the early Drosophila embryo. Elife 6.This study uses an optogenetic approach in Drosophila to examine how shortened or truncated Bicoid exposure affects Bicoid function. The study provides evidence that prolonged exposure to Bicoid is essential for anterior development.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

54. Lin HC, Holland LZ, and Holland ND (2006). Expression of the AmphiTcf gene in amphioxus: insights into the evolution of the TCF/LEF gene family during vertebrate evolution. Dev Dyn 235, 3396–3403. [PubMed: 17013891]

••55. Kozmikova I, and Kozmik Z (2020). Wnt/beta-catenin signaling is an evolutionarily conserved determinant of chordate dorsal organizer. Elife 9.This study examines the role of canonical Wnt signaling in cephalochordates and exposes similarities with vertebrates.

56. Gregor T, Wieschaus EF, McGregor AP, Bialek W, and Tank DW (2007). Stability and nuclear dynamics of the bicoid morphogen gradient. Cell 130, 141–152. [PubMed: 17632061]

••57. Koromila T, Gao F, Iwasaki Y, He P, Pachter L, Gergen JP, and Stathopoulos A (2020). Odd-paired is a pioneer-like factor that coordinates with Zelda to control gene expression in embryos. Elife 9.This study uses ATAC- and ChIP-sequencing approaches in Drosophila to establish a role of Odd-paired in driving chromatin accessibility during early gastrulation of the Drosophila embryo to advance pattern formnation along the dorso-ventral axis.

58. Zaret KS (2020). Pioneer Transcription Factors Initiating Gene Network Changes. Annu Rev Genet 54, 367–385. [PubMed: 32886547]

59. Fernandez Garcia M, Moore CD, Schulz KN, Alberto O, Donague G, Harrison MM, Zhu H, and Zaret KS (2019). Structural Features of Transcription Factors Associating with Nucleosome Binding. Mol Cell 75, 921–932 e926. [PubMed: 31303471]

60. Johnson JL, Georgakilas G, Petrovic J, Kurachi M, Cai S, Harly C, Pear WS, Bhandoola A, Wherry EJ, and Vahedi G (2018). Lineage-Determining Transcription Factor TCF-1 Initiates the Epigenetic Identity of T Cells. Immunity 48, 243–257.e210. [PubMed: 29466756]

61. Onal P, Gunasinghe HI, Umezawa KY, Zheng M, Ling J, Azeez L, Dalmeus A, Tazin T, and Small S (2021). Suboptimal Intermediates Underlie Evolution of the Bicoid Homeodomain. Mol Biol Evol 38, 2179–2190. [PubMed: 33599280]

62. Thorpe CJ, Schlesinger A, Carter JC, and Bowerman B (1997). Wnt signaling polarizes an early C. elegans blastomere to distinguish endoderm from mesoderm. Cell 90, 695–705. [PubMed: 9288749]

63. Freeman G (1990). The establishment and role of polarity during embryogenesis in hydrozoans. In The cellular and molecular biology of pattern formation, Stocum DL and Karr TL, eds. (Oxford University Press).

64. Lee PN, Kumburegama S, Marlow HQ, Martindale MQ, and Wikramanayake AH (2007). Asymmetric developmental potential along the animal-vegetal axis in the anthozoan cnidarian, Nematostella vectensis, is mediated by Dishevelled. Dev Biol 310, 169–186. [PubMed: 17716645]

65. Peng CJ, and Wikramanayake AH (2013). Differential regulation of disheveled in a novel vegetal cortical domain in sea urchin eggs and embryos: implications for the localized activation of canonical Wnt signaling. PLoS One 8, e80693. [PubMed: 24236196]

66. Bolognesi R, Fischer TD, and Brown SJ (2009). Loss of Tc-arrow and canonical Wnt signaling alters posterior morphology and pair-rule gene expression in the short-germ insect, Tribolium castaneum. Dev Genes Evol 219, 369–375. [PubMed: 19705150]

67. Heger P, Zheng W, Rottmann A, Panfilio KA, and Wiehe T (2020). The genetic factors of bilaterian evolution. Elife 9.

68. Philippe H, Poustka AJ, Chiodin M, Hoff KJ, Dessimoz C, Tomiczek B, Schiffer PH, Muller S, Domman D, Horn M, et al. (2019). Mitigating Anticipated Effects of Systematic Errors Supports Sister-Group Relationship between Xenacoelomorpha and Ambulacraria. Curr Biol 29, 1818–1826 e1816. [PubMed: 31104936]

69. Kapli P, Natsidis P, Leite DJ, Fursman M, Jeffrie N, Rahman IA, Philippe H, Copley RR, and Telford MJ (2021). Lack of support for Deuterostomia prompts reinterpretation of the first Bilateria. Sci Adv 7.

70. Kapli P, and Telford MJ (2020). Topology-dependent asymmetry in systematic errors affects phylogenetic placement of Ctenophora and Xenacoelomorpha. Sci Adv 6.

71. Grande C, Martin-Duran JM, Kenny NJ, Truchado-Garcia M, and Hejnol A (2014). Evolution, divergence and loss of the Nodal signalling pathway: new data and a synthesis across the Bilateria. Int J Dev Biol 58, 521–532. [PubMed: 25690967]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript


72. Kenny NJ, Namigai EK, Dearden PK, Hui JH, Grande C, and Shimeld SM (2014). The Lophotrochozoan TGF-β signalling cassette - diversification and conservation in a key signalling pathway. Int J Dev Biol 58, 533–549. [PubMed: 25690968]
73. Wikramanayake AH, Huang L, and Klein WH (1998). beta-Catenin is essential for patterning the maternally specified animal-vegetal axis in the sea urchin embryo. Proc Natl Acad Sci U S A 95, 9343–9348. [PubMed: 9689082]
74. Lapraz F, Haillot E, and Lepage T (2015). A deuterostome origin of the Spemann organiser suggested by Nodal and ADMPs functions in Echinoderms. Nat Commun 6, 8927. [PubMed: 26582589]
75. Lapraz F, Besnardeau L, and Lepage T (2009). Patterning of the dorsal-ventral axis in echinoderms: insights into the evolution of the BMP-chordin signaling network. PLoS Biol 7, e1000248. [PubMed: 19956794]
76. Duboc V, Rottinger E, Besnardeau L, and Lepage T (2004). Nodal and BMP2/4 signaling organizes the oral-aboral axis of the sea urchin embryo. Dev Cell 6, 397–410. [PubMed: 15030762]
77. Darras S, Gerhart J, Terasaki M, Kirschner M, and Lowe CJ (2011). beta-catenin specifies the endomesoderm and defines the posterior organizer of the hemichordate Saccoglossus kowalevskii. Development 138, 959–970. [PubMed: 21303849]
78. Rottinger E, DuBuc TQ, Amiel AR, and Martindale MQ (2015). Nodal signaling is required for mesodermal and ventral but not for dorsal fates in the indirect developing hemichordate, Ptychodera flava. Biol Open 4, 830–842. [PubMed: 25979707]
79. Wlizla M (2011). Evolution of nodal signaling in deuterostomes: insights from Saccoglossus kowalewskii. In Organimsmal Biology and Anatomy, Volume PhD. (Chicago: University of Chicago).
80. Darras S, Fritzenwanker JH, Uhlinger KR, Farrelly E, Pani AM, Hurley IA, Norris RP, Osovitz M, Terasaki M, Wu M, et al. (2018). Anteroposterior axis patterning by early canonical Wnt signaling during hemichordate development. PLoS Biol 16, e2003698. [PubMed: 29337984]
81. Onai T, Yu JK, Blitz IL, Cho KW, and Holland LZ (2010). Opposing Nodal/Vg1 and BMP signals mediate axial patterning in embryos of the basal chordate amphioxus. Dev Biol 344, 377–389. [PubMed: 20488174]
82. Pelliccia JL, Jindal GA, and Burdine RD (2017). Gdf3 is required for robust Nodal signaling during germ layer formation and left-right patterning. Elife 6.
83. Montague TG, and Schier AF (2017). Vg1-Nodal heterodimers are the endogenous inducers of mesendoderm. Elife 6.
84. Arendt D, and Nubler-Jung K (1994). Inversion of dorsoventral axis? Nature 371, 26. [PubMed: 8072524]
85. Nubler-Jung K, and Arendt D (1994). Is ventral in insects dorsal in vertebrates? : A history of embryological arguments favouring axis inversion in chordate ancestors. Rouxs Arch Dev Biol 203, 357–366. [PubMed: 28305940]
86. Holland LZ, and Onai T (2012). Early development of cephalochordates (amphioxus). Wires Dev Biol 1, 167–183.
87. Winkley KM, Kourakis MJ, DeTomaso AW, Veeman MT, and Smith WC (2020). Tunicate gastrulation. Curr Top Dev Biol 136, 219–242. [PubMed: 31959289]
88. Osada SI, and Wright CV (1999). Xenopus nodal-related signaling is essential for mesendodermal patterning during early embryogenesis. Development 126, 3229–3240. [PubMed: 10375512]
89. Hatschek B (1893). The amphioxus and its development (Tuckey James, transl. and ed.), (London: Swan, Sonnenschein & co.).
90. Kaji T, Reimer JD, Morov AR, Kuratani S, and Yasui K (2016). Amphioxus mouth after dorso-ventral inversion. Zool Lett 2.
91. Veeman MT, Newman-Smith E, El-Nachef D, and Smith WC (2010). The ascidian mouth opening is derived from the anterior neuropore: Reassessing the mouth/neural tube relationship in chordate evolution. Developmental Biology 344, 138–149. [PubMed: 20438724]
92. Dickinson A, and Sive H (2007). Positioning the extreme anterior in Xenopus: Cement gland, primary mouth and anterior pituitary. Semin Cell Dev Biol 18, 525–533. [PubMed: 17509913]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

93. Fischer A, Hofmann DK, and Dorresteijn A (2013). Developmental Biology of the “Spiralia”. In The Helgoland manual of animal development, Fischer A, ed. (Munich: Verlag Dr. Friedrich Pfeil), pp. 128–169.

94. Henry JQ, Perry KJ, Wever J, Seaver E, and Martindale MQ (2008). Beta-catenin is required for the establishment of vegetal embryonic fates in the nemertean, Cerebratulus lacteus. Dev Biol 317, 368–379. [PubMed: 18387602]

95. Henry JQ, Perry KJ, and Martindale MQ (2010). Beta-catenin and early development in the gastropod, Crepidula fornicata. Integr Comp Biol 50, 707–719. [PubMed: 21558234]

96. Lanza AR, and Seaver EC (2020). Activin/Nodal signaling mediates dorsal-ventral axis formation before third quartet formation in embryos of the annelid Chaetopterus pergamentaceus. Evodevo 11, 17. [PubMed: 32788949]

97. Lanza AR, and Seaver EC (2020). Functional evidence that Activin/Nodal signaling is required for establishing the dorsal-ventral axis in the annelid Capitella teleta. Development 147.

98. Lanza AR, and Seaver EC (2018). An organizing role for the TGF-β signaling pathway in axes formation of the annelid Capitella teleta. Dev Biol 435, 26–40. [PubMed: 29337130]

99. Grande C, and Patel NH (2009). Nodal signalling is involved in left-right asymmetry in snails. Nature 457, 1007–1011. [PubMed: 19098895]

100. Kuroda R, Endo B, Abe M, and Shimizu M (2009). Chiral blastomere arrangement dictates zygotic left-right asymmetry pathway in snails. Nature 462, 790–794. [PubMed: 19940849]

101. Reversade B, and De Robertis EM (2005). Regulation of ADMP and BMP2/4/7 at opposite embryonic poles generates a self-regulating morphogenetic field. Cell 123, 1147–1160. [PubMed: 16360041]

102. Lee HX, Ambrosio AL, Reversade B, and De Robertis EM (2006). Embryonic dorsal-ventral signaling: secreted frizzled-related proteins as inhibitors of tolloid proteinases. Cell 124, 147–159. [PubMed: 16413488]

103. Lambert JD, Johnson AB, Hudson CN, and Chan A (2016). Dpp/BMP2–4 Mediates Signaling from the D-Quadrant Organizer in a Spiralian Embryo. Curr Biol 26, 2003–2010. [PubMed: 27397892]

104. Lyons DC, Perry KJ, Batzel G, and Henry JQ (2020). BMP signaling plays a role in anterior-neural/head development, but not organizer activity, in the gastropod Crepidula fornicata. Dev Biol 463, 135–157. [PubMed: 32389712]

105. Martin-Duran JM, Passamaneck YJ, Martindale MQ, and Hejnol A (2016). The developmental basis for the recurrent evolution of deuterostomy and protostomy. Nat Ecol Evol 1, 5. [PubMed: 28812551]

••106. Larkin A, Marygold SJ, Antonazzo G, Attrill H, Dos Santos G, Garapati PV, Goodman JL, Gramates LS, Millburn G, Strelets VB, et al. (2021). FlyBase: updates to the Drosophila melanogaster knowledge base. Nucleic Acids Res 49, D899–D907. [PubMed: 33219682] This paper describes the most important online resource for insect research, which is currently under threat of losing critical funding.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

**Box 1:**

## Selection of embryological features that obscure the shared metazoan heritage of axis specification mechanisms

1. In cnidarians (jelly fish) and ctenophores (comb jellies), the posterior end of the embryo forms at the site of polar body expulsion. However, in bilaterians, the polar bodies (abortive meiotic nuclei) give rise to anterior (apical) structures and thus by convention, defines the animal pole. The mechanism for linking posterior specification and polar body expulsion in cnidarians and ctenophores is unknown, but it reflects the position of the oocyte and zygote nucleus [63]. As shown in the sea anemone *Nematostella vectensis*, the nuclear position coincides with the side of Dishevelled protein accumulation, which in turn may interfere with the ß-catenin destruction complex [64].

2. In most deuterostomes, it is difficult to untangle the roles of ß-catenin and Nodal signaling in primary (animal-vegetal) and secondary (dorsoventral) axis specification. Nodal signaling is an evolutionary novelty of the Bilateria [67], the last common ancestor of which may have been very similar to the last common ancestor of deuterostomes [68–70]. Nodal signaling functions in parallel with the BMP pathway through paralogous receptor and effector proteins [67, 71, 72]. In non-chordate deuterostomes, such as sea urchins, the roles of ß-catenin, Nodal, and BMP signaling can be discerned more readily than in other deuterostomes. In these species, ß-catenin specifies endomesoderm and gastrulation at the vegetal (posterior) pole, breaking symmetry along the primary axis [9, 10, 73], and Nodal specifies the oral ectoderm, thereby breaking symmetry along the secondary axis and dissociating the formation of the mouth from the site of gastrulation (deuterostomy) [74–76]. Due to Nodal-dependent expression of the BMP antagonist Chordin on the oral side, Nodal also establishes an aboral-to-oral (dorsal-to-ventral) gradient of BMP activity, i.e., the entire secondary axis of sea urchin embryos. The related hemichordates also use ß-catenin for establishing the primary body axis [77] and Nodal for specifying chordin expressing oral ectoderm [78], but the gene network differs especially in direct developers with delayed specification of the oral ectoderm [79]. Moreover, hemichordates also use ß-catenin for posterior extension of the embryo [80]. In chordates, the concerted actions of ß-catenin and Nodal in primary and secondary axis specification are even more difficult to untangle. For example, in cephalochordates [55, 81] and vertebrates [6, 82, 83], the two molecules function interdependently in primary and secondary axis specification.

3. The terms dorsal and ventral are used for opposite body sides in chordate and non-chordate bilaterians [84, 85]. This is probably a consequence of the evolution new mouth openings in chordates and their relocation to the formerly aboral body side. Chordate innovations in gastrulation, including

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

the infolding ("mesodermalization") of the *nodal* and *chordin*-expressing organizer of the secondary body axis, and neurulation [86–88], preclude Nodal-dependent deuterostomy, as seen in echinoderms and hemichordates and are incompatible with the blastopore becoming the mouth (protostomy), as seen in cnidarians and ctenophores, because neurulation covers the blastopore [89]. Cephalochordates resolved this issue by unilaterally fusing somatic mesoderm with adjacent ectoderm and endoderm. During subsequent developmental stages, cephalochordates move this new oral opening away from the neural fold to the opposite (formerly aboral) body side [90], possibly in response to their filter-feeding behavior in sandy grounds. In the lineage of urochordates and vertebrates, a new oral opening evolved anterior to the neural fold [91, 92], from where the increasing size of the vertebrate brain may have pushed it anterior and then to the opposite body side. In summary, cephalochordates and vertebrates independently relocated their oral openings to the formerly aboral side, referred to as the chordate's "ventral" side. This convention not only confounds dorsal and ventral but also left and right when comparing the body plans of chordate and non-chordate bilaterians, as reviewed elsewhere [71].

4. Stereotypic asymmetric early cleavage patterns led to modifications in axial patterning in many animal groups (e.g., nematodes, urochordates, spiralians). Superphylum of spiralians, annelids, mollusks, ribbon worms, and free-living flatworms share a stereotypic cleavage pattern called spiral cleavage. The hallmark of spiral cleavage is that the first two (equal or unequal) cell divisions produce four blastomeres (quadrants) that undergo synchronous cleavage cycles with alternating oblique spindle orientations along the primary (animal-vegetal) body axis [93]. While ß-catenin establishes the vegetal (posterior) pole of the larva in ribbon worms (Nemertini) [94], other spiralians have modified this mechanism in ways that remain poorly understood [95]. Additionally, spiralians use a specific blastomere lineage as organizer of the secondary axis. This cell lineage is determined by asymmetric inheritance of maternal material and/or induction (reviewed in [96, 97]). While Nodal signaling is required for this process, directly or indirectly, in distantly related polychaetes (Annelida) [96–98], it is not required in snails (Mollusca) [99, 100]. When compared to deuterostomes, the organizers for the secondary axis in animals with spiralia cleavage differ in two important ways. First, they are established on non-homologous body sides (aboral/dorsal in spiralians and oral/ventral in deuterostomes (called "dorsal" in chordates; see previous section). Second, while the deuterostome organizer expresses the BMP antagonist Chordin and is characterized by reduced BMP activity [101, 102], the spiralian organizer lacks Chordin and exhibits peak BMP activity in some species[103] or does not require BMP signaling for specifying the secondary body axis [104]. Brachiopods, which may have reverted from spiral to radial cleavage, use ß-catenin and BMP activity (but not Nodal [71]) to promote posterior and dorsal cell fate specification, respectively, possibly in conjunction with additional factors

that remain to be discovered [105]. In conclusion, stereotypic asymmetric cleavage patterns in species with spiral cleavage enabled lineage-specific modifications in the primary and secondary axis specification mechanisms, including the loss of organizer activity on the oral/ventral body side.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

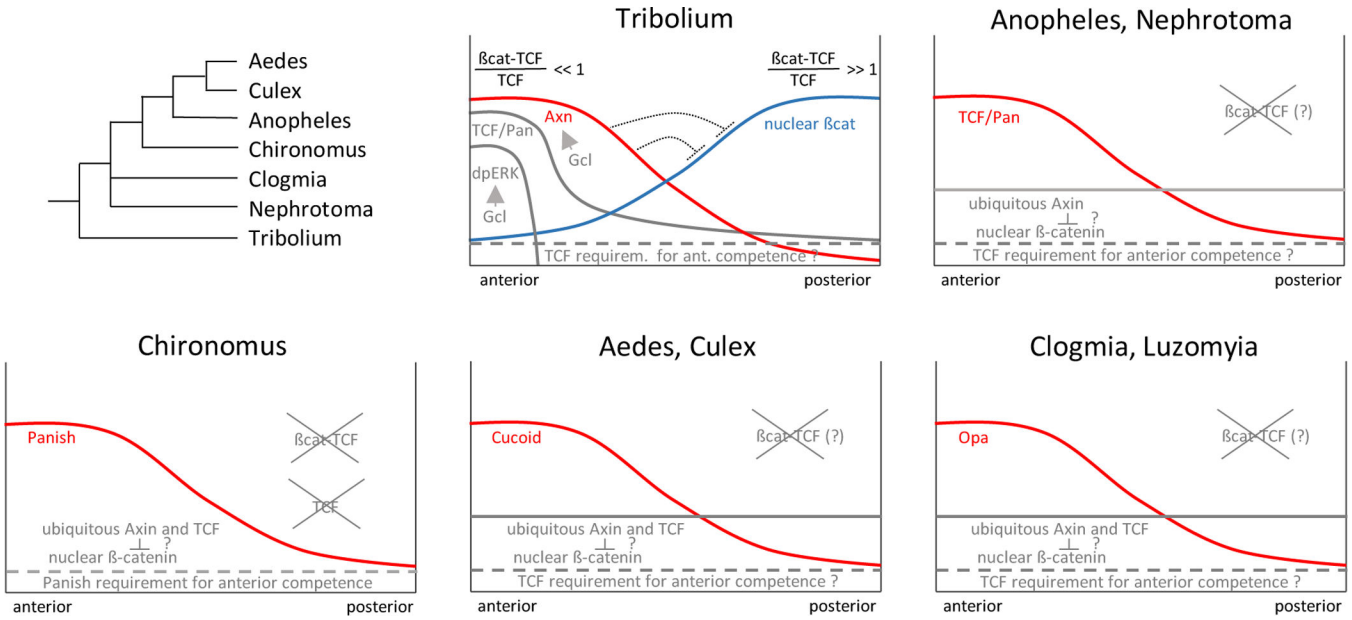


**Figure 1.**

Inferred maternal activities of ß-catenin and TCF in early embryos of the beetle *Tribolium castaneum* and various midges and mosquitoes. Note that Chironomus and Drosophila (not shown) lack maternal Tcf expression. Protein activities (y-axis) are inferred based on maternal transcript distributions and knockdown experiments, as described in the text.



Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

**Table 1.**

Presence (+) and absence (−) of maternal transcripts and posterior germ plasm in dipteran eggs[36, 37, 106]. Functionally validated (bold) or predicted (based on anterior-posterior expression profiling) anterior determinants (ADs) are indicated for Tipulomorpha (green), chironomid Culicomorpha (yellow), culicid Culicomoprha (orange), Psychodomorpha (blue), and Cyclorrhapha (grey) representatives. The gene list includes *bicoid* (*bcd*), *odd-paired* (*zic/opa*), *cucoid*, *pangolin* (*Tcf/pan*), *panish*, *wnt* family (*wnt*), the Wnt receptor complex genes *frizzled* (*fz*) and *arrow* (*LRP5–6/arr*), and genes that encode key regulators of intracellular ß-catenin levels, including *dishevelled* (*dsh*), *axin* (*axn*), *APC-like* (*apc)*, and *shaggy* (gsk3ß/*sgg*).

| | **Nephrotoma** | **Chironomus** | **Anopheles** | **Aedes** | **Culex** | **Clogmia** | **Lutzomyia** | **Drosophila** |
|---|---|---|---|---|---|---|---|---|
| *Tcf/pan* | AD | − | **AD** | + | + | + | + | − |
| *panish* | not appl. | **AD** | not appl. | not appl. | not appl. | not appl. | not appl. | not appl. |
| *cucoid* | + | + | + | **AD** | **AD** | + | + | n. a. |
| *zic/opa* | + | + | + | + | + | **AD** | AD | − |
| *bcd* | not appl. | not appl. | not appl. | not appl. | not appl. | not appl. | not appl. | **AD** |
| | | | | | | | | |
| *wnt* | + | − | − | + | + | − | (−) | − |
| *fz* | + | + | + | + | + | + | + | + |
| *LRP5–6/arr* | + | + | + | + | + | + | + | (−) |
| *dsh* | + | + | + | + | + | + | + | + |
| *axn* | + | + | + | + | + | + | + | + |
| *apc* | + | + | + | + | + | + | + | + |
| *gsk3ß/sgg* | + | + | + | + | + | + | + | + |
| *ß-catenin/arm* | + | + | + | + | + | + | + | + |
| | | | | | | | | |
| germ plasm | + | + | + | + | + | − | + | + |