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Deconstructing the molecular mechanisms shaping the vertebrate body plan

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The large display of body shapes and sizes observed among vertebrates ultimately represent variations of a common basic body plan. This likely results from the use of homologous developmental schemes, just differentially tinkered both in amplitude and timing by natural selection. In this review, we will revisit, discuss and combine old ideas with new concepts to update our view on how the vertebrate body is built. Recent advances, particularly at the molecular level, will guide our deconstruction of the individual developmental modules that sequentially produce head, neck, trunk and tail structures, and the transitions between them.

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Introduction

Formation of the vertebrate body is a dynamic and complex process that starts at the end of gastrulation. Progressively, from head to tail, the body axis is laid down by the sequential addition of primodiae for the different body structures at the posterior embryonic end [1]. This process can be divided into four sequential stages. The first stage comprises production of head structures, essentially formed by the brain and most sensory organs encased in a rigid skull. After completion of head structures, a profound switch in developmental mechanisms leads to the formation of the trunk, which contains the largest part of the respiratory, digestive, reproductive and excretory organs. These are held and protected by a rib cage and a mobile axial skeleton composed of vertebrae that also enclose the spinal cord. The neck emerges as a transitory region between the head and the trunk, already containing spinal cord and vertebral column but still not associated with major organic systems. The last stage of vertebrate body formation is tail development, which yields a muscle-skeletal structure involved in locomotion, balance, defence and intraspecific communication. In some species, such as in humans, tail development has been modified in such a way that only a vestigial element is left [2,3].

The head, neck and trunk all rely on the epiblast to produce their neural tube and require the activity of the Primitive Streak (PS) to generate mesodermal components. This contrasts with tail development, which depends on the tail bud to build both its mesoderm and neural tube [4]. These differences were initially observed by Holmdahl almost 100 years ago, coining the terms primary and secondary body for PS-dependent and tail bud-dependent regions, respectively [5]. Today, recent advances enable us to address the formation of the vertebrate body at a molecular level, thus expanding Holmdahl's classical vision.

New layers in the regulation of PS formation

Formation of the PS is a key step in vertebrate development. This is a complex process involving a variety of cellular and molecular interactions limiting a rather broad cellular competence to a specific area of a morphogenetic field [6]. Recent findings have discovered extra regulatory layers to this process. Classic genetic experiments had shown that the Transforming Growth Factor-β (TGF-β) family member Nodal (See Box 1) is a central component of the molecular network inducing PS formation [6]. However, the pre-gastrulating amniote embryo contains Nodal activity scattered throughout the epiblast. Nevertheless, this activity is unable to trigger gastrulation because it is kept below a critical threshold level by specific inhibitors, including the Cerl1 or the Lefty proteins [7]. Yet, it still allows for sporadic cell delamination throughout the epiblast [8]. It has now been shown that this scattered activity converges in a restricted domain as a consequence of stereotyped cell movements within this epithelial layer. This convergence triggers a positive feedback loop that promotes a local increase of Nodal signalling and of Crumbs2-dependent stimulation of Epithelial to Mesenchymal Transition (EMT)-associated processes, confining and amplifying the cell delaminating activity in that area of the epiblast to generate the PS [8,9•]. Rather surprisingly, it was recently found that regulation of *Lefty* expression involves an epigenetic mechanism, requiring the activity of the *Tet* genes to keep their promoters non-methylated. Indeed, in *Tet* mutant embryos Nodal activity becomes excessively high throughout the epiblast, leading to the formation of ectopic PSs [10••].

Box 1 .

Cdx genes

Cdx homeobox genes belong to the *ParaHox* gene cluster and encode several important transcription factors that control axial patterning.

Cerl1

This gene encodes a cytokine and functions as an antagonist of the TGF-β family. It is involved in vertebrate head and heart induction and in the formation and patterning of the PS.

Crumbs2

This gene encodes for a member of the Crumbs cell polarity complex family of proteins. Recent data suggests that, in mammals, *Crumbs2* is necessary to remove, rather than maintain, apical junctions. It plays a key role in mesoderm production by completing the EMT of epiblast cells in the PS.

Gdf11

Gdf11 belongs to the TGF-β superfamily of proteins. It binds to the growth factor-β receptor Alk5, regulating the expression of key genes (e.g. *Hox* genes) during embryonic development. It is involved in the Anterior-Posterior (AP) patterning, particularly during tail bud and tail formation.

***Lefty* genes**

Lefty genes encode antagonists of Nodal activity, by preventing the interaction between Nodal and its receptors. *Lefty-1* is crucial during gastrulation, confining Nodal activity to the future PS region. It is also involved the establishment of left-right asymmetry.

Nodal

Nodal is a signaling molecule of the TGF-β superfamily. Binding of Nodal to specific surface receptors triggers a signaling pathway involving recruitment and activation of the SMAD transcription factors family. It plays key roles in PS formation and in the establishment of left-right symmetry.

Oct4 (Pou5f1)

Embryonic stem cells are governed by a core of key transcription factors including Oct4. This gene encodes a protein essential for pluripotency and self-renewal properties during embryonic development. Additionally, it has recently been shown to play a key role controlling vertebrate trunk length.

Sox2

Sox2 is a member of the SRY-related HMG-box (SOX) family of transcription factors that plays critical roles in a variety of developmental processes. It is involved in maintaining the self-renewal of neural progenitor cells, thus playing a key role in neural tube formation.

T

The T-box transcription factor *Brachyury* (*T*) plays several key roles during embryonic development, particularly in mesoderm formation and differentiation by transcriptional regulation of important mesoderm-associated genes. It is also crucial for notochord development.

***Tet* genes**

Tet genes encode for proteins of the TET (ten-eleven translocation) family that catalyse DNA demethylation and control gene expression through epigenetic mechanisms.

Wnt3/Wnt3a

Wnt proteins are involved in key intercellular signalling with multiple crucial roles during embryonic development. Wnt3 regulates AP patterning in the early embryo and PS formation. Wnt3a is necessary for mesoderm production, as it controls progenitor cell fate during axial elongation.

The neuro-mesodermal progenitors as a common theme in post-cranial body formation

Head, neck and trunk have been classified as primary body on the basis of common general developmental features. However, there are fundamental mechanistic differences between the production of head and post-cranial vertebrate structures (i.e. neck, trunk and tail) [4]. Two particularly relevant indicators of this change are the control of PS activity, that becomes independent of Nodal signalling [11], and the drastic modification in the building of body structures. In particular, while the primordiae for the head-associated neural and mesodermal structures are mostly shaped concomitantly with the appearance and early organizing activity of the PS, the postcranial body is progressively laid down as the embryo extends at its caudal end. In the neural tube and somitic mesoderm, this developmental switch marks the outset of the spinal cord and vertebral column and is functionally linked to the appearance of the Neuro-Mesodermal Progenitor (NMP) [12]. This cell population was identified in grafting experiments by its ability to generate neural and paraxial mesodermal tissues throughout development [13,14], and further characterized by retrospective clonal analyses in the mouse embryo [15]. Despite some controversy, it is generally accepted that NMPs are the origin of the spinal cord and paraxial (somitic) mesoderm found in the neck, trunk and tail structures, thus contributing to both primary and secondary body structures [12,15]. In amniote embryos, NMPs involved in neck and trunk development are located in two specific areas of the epiblast, the node-streak border and the anterior part of the caudal lateral epiblast [14,16•]. These cells are then reallocated to the Chordo-Neural Hinge (CNH), within the tail bud, when the embryo engages in secondary body formation [14]. The observation that the CNH contains remnants of the blastopore lip in amphibians and the node in amniotes [17,18] suggests a mechanistic continuity between primary and secondary body formation, which was further supported by retrospective clonal analyses revealing that tail bud NMPs are direct derivatives of their trunk counterparts [15]. Based in the preeminent role that NMPs have during vertebrate development we, therefore, propose that, in addition to primary and secondary body, vertebrate development could be further divided in NMP-independent and NMP-dependent stages.

NMPs are currently defined by their co-expression of Brachyury (T) and Sox2, mesodermal and neural markers, respectively [14,16•]. These cells are thus thought to be in a transient state between these two lineages, and their ultimate choice resulting from the balance between the networks controlling each fate. A variety of *in vitro* and *in vivo* experiments are consistent with this idea and have also identified Wnt3a as a key regulator of lineage choice in these cells. Collectively, these studies show that NMPs take neural routes when exposed to low or negative Wnt3a activity and mesodermal fates upon increased and sustained

Wnt3a activity, which is further promoted and stabilized by Tbx6 and Msgn1 [19,20,21•,22••,23–25,26•,27–29].

NMPs are involved in the formation of primary and secondary body structures. Some factors, like T, Wnt3a, Cdx, and Fgf signalling regulate their activity at all axial levels [19,30,31,32•,33,34]. However, recent studies indicate that genetic networks regulating early trunk and early tail progenitors in the mouse seem to be fundamentally different, relying on distinctive subsets of genes for trunk and tail formation [22••]. Among them, *Oct4* stands out as a key regulator of trunk development, being dispensable at tail bud stages. Indeed, conditional *Oct4* inactivation after completion of its role during early pluripotent stages produced embryos lacking trunk structures, but that still contained recognizable tail bud derivatives [35]. Conversely, sustained *Oct4* activity in the epiblast was sufficient to extend formation of trunk structures, maintaining typical primary body growth characteristics, as well as delaying or even completely blocking the transition into secondary body formation [36••]. The importance of Oct4 for trunk development was further supported by the observation that in snake embryos, characterized by extremely long trunks, recombination events most likely brought *Oct4* under the control of regulatory elements that kept its expression active for exceptionally long developmental times [36••].

Similar structures, different mechanisms

Somites are another key element of vertebrate development. Interestingly, although they are produced throughout most of the main body axis, the mechanisms regulating their formation vary at different axial levels. The first few somites, which form the occipital bone instead of vertebrae [37], are not built following the general model of somitogenesis, as they are particularly resilient to genetic alterations that strongly compromise the development of more caudal somites [38,39]. Interestingly, recent observations indicate that formation of NMP-derived somites is also mechanistically different during primary and secondary body formation. In particular, while complete inactivation of *Lfng* interferes globally with postcranial somitogenesis [40,41], oscillatory expression of this gene is required to build trunk somites but dispensable at tail levels [42]. Tail somites were also shown to be more resilient to reductions in *Lfng* dosage than their trunk counterparts [43]. Conversely, *Hoxb6* readily interferes with somite formation at tail levels but have no negative effects on the same process in more anterior regions, further supporting mechanistic differences in somitogenesis during primary and secondary body formation [44].

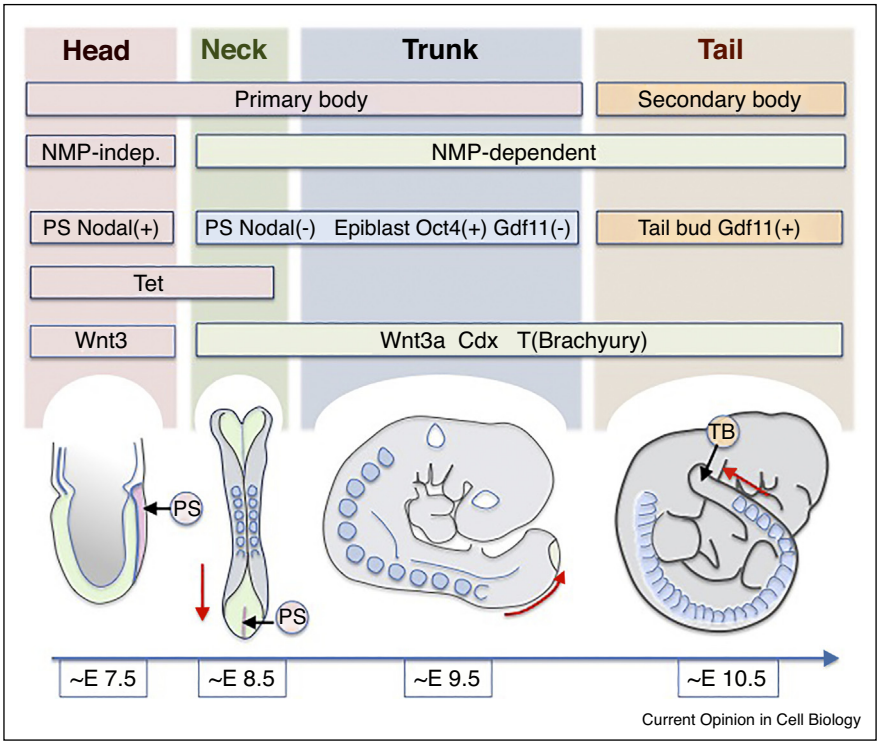
Regulating transitions between compartments

Based on the above discussion, vertebrate development seems to require at least two major functional transitions: from NMP-independent to NMP-dependent and from primary to secondary body formation (see Figure 1). The first transition entails a switch in canonical Wnt signalling from requiring *Wnt3* at head stages to rely on *Wnt3a* when entering the NMP-dependent phase [[32•],19]. The mechanisms underlying this change are not known. However, in mouse embryos carrying a stabilized version of the Axin2 protein, Wnt signalling was reduced during the *Wnt3* phase but stimulated after switching to the *Wnt3a*-driven stage [45], indicating that the *Wnt3* to *Wnt3a* swap is associated with a mechanistic change in the canonical Wnt pathway. During this first transition, axial extension also becomes dependent on *T* and the *Cdx* genes [31,32•,46]. *Wnt3a*, *T* and *Cdx* activities might indeed cooperate to promote the emergence of NMPs as recent genomic studies revealed the opening of key enhancers upon Wnt-induced NMP formation, followed by cooperative binding of T and Cdx proteins to regulatory regions of Wnt and Fgf signalling targets involved in NMP activity [32•].

The transition from head to neck/trunk development also requires changing the developmental potential of epiblast cells fated to intermediate and lateral mesoderm. During head formation, these cells generate the heart primordion, whereas in the trunk they contribute to a wide variety of organs of the digestive, excretory and reproductive systems. Recent data indicates that a signal from the NMP-derived neural tube is instrumental to prevent the production of heart lineages from the lateral mesoderm. Interestingly, as for PS formation, Tet-mediated epigenetic modifications seem to play an essential role in this process [47••]. Specifically, in the absence of Tet activity, Wnt signalling becomes over-activated in the progenitor zone, blocking neural differentiation of NMPs, which in turn leads to a failure in keeping the cardiac field within its normal domain, extending caudally into the prospective neck region [47••]. These observations indicate that the position of the first vertebrae and the caudal limit of the heart lineages are both inter-related and linked to the emergence of NMPs, which occurs at an almost invariable axial position in different vertebrate species. Conversely, activation of trunk lateral mesoderm, generally marked by the position of the forelimb bud, occurs at highly variable axial levels. The relative timing of these two processes most likely will provide the basis for the broad neck length diversity among vertebrate species.

As for the last transition, recent data places Gdf11 signalling at the top of the regulatory hierarchy controlling the shift from primary to secondary body formation. The absence of *Gdf11* alone or together with *Gdf8* resulted in fetuses with extended trunks [48,49], whereas premature activation of Gdf11 signalling led to dramatic shortenings in this region, with an early transition into secondary body formation [50]. The axial level of Gdf11 expression directly correlates with the position of the transition into tail development in a variety of vertebrate

Figure 1



Schematic representation of the different sequential stages involved in the formation of the vertebrate body. The head, neck and trunk represent the primary body, associated with the presence of the primitive streak (PS), whereas the tail represents the secondary body, relying on the tail bud (TB). Not all primary body depends on the same mechanisms. In the head, the PS requires Nodal and Wnt3 but in the trunk the PS is independent of these signals, becoming dependent on Oct4. The neck, trunk and tail all require NMPs for their development, which coincides with the requirement of T, Wnt3a and the Cdx family for their development. The drawings represent mouse embryos at stages related to the production of head, neck, trunk and tail structures. The red arrow indicates the direction of the axial growth.

species [36••,51], further supporting a key role for Gdf11 signalling in this process. Interestingly, Gdf11 and Nodal signalling share many features, which constitute an interesting parallelism between mechanisms initiating and finishing PS-dependent vertebrate development. Nonetheless, the mechanisms operating downstream of Gdf11 signalling are only partially understood. One of the key components of this process, the end of organ-producing progenitors, seems to depend on the Gdf11-mediated activation of *Isl1* in the progenitors for the intermediate and lateral mesoderm [50]. This activation triggers the terminal differentiation of those progenitors by means of inducing both the hindlimb from its somatic layer and the cloaca-associated mesoderm from its splanchnic component. However, the mechanisms regulating NMP reallocation from the epiblast into the tail bud remain to be discovered.

Concluding remarks

Holmdahl's first proposal for the existence of primary and secondary body formation was solely based on morphological traits. The age of genetics has mostly proven him right; yet, it also revealed the enormous complexity surrounding the development of the vertebrate body. Despite its continuous nature, the process of axis extension seems to be organized into developmental units, each controlled by distinctive molecular networks that broadly correspond to each body region. Recent data has added new layers of complexity to these relevant regulatory networks, including the discovery of acting epigenetic mechanisms. A full understanding of these processes and their mutual interactions will further our knowledge of how the vertebrate body plan is shaped and how modifications in these processes and interactions produced the wide variety of body shapes displayed among vertebrate species.

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