Somite development and regionalisation of the vertebral axial skeleton

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Abstract

A critical stage in the development of all vertebrate embryos is the generation of the body plan and its subsequent patterning and regionalisation along the main anterior-posterior axis. This includes the formation of the vertebral axial skeleton. Its organisation begins during early embryonic development with the periodic formation of paired blocks of mesoderm tissue called somites. Here, we review axial patterning of somites, with a focus on studies using amniote model systems – avian and mouse. We summarise the molecular and cellular mechanisms that generate paraxial mesoderm and review how the different anatomical regions of the vertebral column acquire their specific identity and thus shape the body plan. We also discuss the generation of organoids and embryo-like structures from embryonic stem cells, which provide insights regarding axis formation and promise to be useful for disease modelling.

Keywords: somite patterning, axis formation, hox genes, axial progenitors, trunk-like structures

1. Introduction

The axial skeleton is perhaps the most obvious example of segmental organization in the vertebrate body. It is composed of a series of repeating units, vertebral bones and ribs, interconnecting joints and ligaments, which are regionalised into the neck, trunk and tail [1, 2]. A major function of the axial skeleton is to provide support for the head and protection for the spinal cord and organs in the ventral body cavity. It provides a surface for the attachment of skeletal muscles that move the head, neck, and trunk, support respiration, and stabilize the proximal parts of the appendicular skeleton – the forelimbs and hindlimbs, via the shoulder and pelvic girdles. Thus, it is critical for vertebrate development. Modifications of the axial skeleton have facilitated variation and evolutionary adaptations amongst vertebrate species [2, 3]. For example, in avian species it has been shown that neck length scales isometrically with leg length, suggesting correlated evolution of these modules [4]. The same group used comparative dissections to examine the craniocervical anatomy in three vulture species with different feeding behaviours. This showed different numbers of cervical vertebrae and variations in the morphology of the atlas-axis complex and the associated neck musculature [5]. Studies of axial morphology are not limited to living species and a recent analysis of the skeleton of an extinct crocodilian found variations to both shoulder and pelvic girdles with additional sacral vertebrae, which may hint at the species becoming more upright [6, 7]. However, our knowledge regarding the embryonic development of these structures comes from studies in model systems, including avian and mice, which can be live imaged and genetically manipulated.

2. Generation and patterning of somites

The vertebral axial skeleton is derived from somites [8], which form sequentially in a rostro caudal progression as paired blocks of paraxial mesoderm on either side of the neural tube. Their formation is governed by the segmentation clock: periodic patterns of oscillating gene expression, which are subject to Retinoic Acid (RA), Wnt and Fibroblast Growth Factor (FGF) signals, creating opposing gradients across presegmented mesoderm (PSM) and somites [1]. The segmentation clock has been

well-characterized in chick, mouse and zebrafish and can be recapitulated in human embryonic stem cell derived PSM [9]. Segmentation is coordinated with embryo growth at the posterior end [10]. Furthermore, the emergence of PSM overlaps with neurulation and neural folds gathering at the axial midline. Elongation of PSM and axial neural tissue is coordinated by inter-tissue forces and mechanical interactions, acting in a feed-back loop to synchronise rates of tissue morphogenesis [11]. During body axis extension, mesenchymal paraxial mesoderm cells display a posterior to anterior gradient of random cell motility. This is downstream of FGF signaling [12], which establishes different metabolic states along the PSM important for regulating these cell behaviours [13].

From late gastrulation, as the primitive streak regresses, the PSM separates into cell aggregates with the first somite apparent at Hamburger-Hamilton (HH) stage 7 (~24 hrs) in chick and embryonic days E7.5-8.0 in mice. The gradual increase in cell density along the PSM [12] is followed by cell shape changes, reorganization of the extracellular matrix, a mesenchymal-epithelial transition and formation of near spherical epithelial somites [14, 15]. Epithelial somites grow rapidly and generate different compartments. Cell behaviours underlying the morphological transitions during somite differentiation have been examined using chick embryos expressing membrane-GFP. This showed somite cells actively explore their environment and contact the surface ectoderm via filopodia-like protrusions. Retrograde transport of the Wnt receptor, Frizzled-7, was observed in these protrusions, indicating they are involved in signal reception [16]. Quantitative image analysis revealed heterogeneity of cell size and proliferation rates across epithelial, maturing and differentiating somites. Within maturing somites, cells displayed directed cellular motion towards medial somite regions, which could result from mechanical constraints imposed by flanking tissues and may contribute to somite compartmentalisation [17].

Work from many groups has shown that, in response to signals from adjacent tissues, somites are patterned along the dorso-ventral, medio-lateral and anterior-posterior axes and generate different compartments. These comprise progenitor cells which adopt different fates and give rise to distinct lineages, eventually differentiating into dermis, skeletal muscle, tendons and cartilage of the vertebrae

and ribs (reviewed in [8, 18-21]). The sclerotome compartment forms by de-epithelialization of the ventral portion of the epithelial somite and notochord-derived Shh induces expression of chondrogenic transcription factors [22-25]. Together with the notochord the sclerotome generates the major components of the intervertebral discs and the vertebral bodies of the spinal column [26]. The dorsal portion of the somite, now known as the dermomyotome, remains epithelial [20, 21, 27]. Cells delaminate from the dermomyotome edges, a process initiated at the anterior-medial margin of the dermomyotome where apical junctions first dissolve [17]. Subsequently all four edges of the dermomyotome are involved, with cells translocating underneath to form the myotome, sandwiched between the remaining dermatome and sclerotome [28]. At the medial lip of the dermomyotome, Notch-Delta mediated interactions between passing neural crest cells and myoblast progenitors trigger their transition into the myotome [29], this involves the stabilization of Snail1 [30]. In response to Wnt11, myoblasts elongate parallel to the neural tube [31] and fuse to generate multinucleated myofibres [32], a process regulated by TGFβ signalling [33]. Myogenic regulatory factors activate myogenic differentiation, with several microRNAs ensuring robustness of this programme through feed-back regulation and fine-tuning [34-37]. In response to myotome derived FGF signalling, sclerotome cells give rise to tendon progenitors expressing the transcription factor scleraxis [38].

The commitment to specific cell lineages occurs relatively late and grafting experiments in chick embryos (reviewed in [39]) showed that at epithelial somite stages cells remain plastic. By contrast, anterior-posterior axial identity of paraxial mesoderm is determined prior to somite formation [40]. This is regulated through the expression of Hox genes (see section 4). How Hox genes interact with somite patterning pathways and cellular differentiation programmes to elaborate different vertebral morphologies and discrete features of associated muscles and connective tissues remains incompletely understood.

3. Regionalisation of the body axis

Somites are initially similar in morphology, but they produce anatomical features characteristic for their position along the body axis. Skeletal elements include vertebrae with complex morphology [39], as well as scapulae and clavicles comprising the forelimb girdle, and pelvic bones comprising the hindlimb girdle. Based on these features, the axis is partitioned into six domains: occipital, cervical, thoracic, lumbar, sacral and caudal (Figure 1A, B). Transition from cervical to thoracic domains is associated with emergence of forelimbs, while the lumbar-sacral transition is associated with emergence of hindlimbs.

Within species the number of vertebrae in each domain is fixed, but between species the number of vertebrae in each domain varies, generating a species-specific axial formula [41]. The occipital domain forms the bony structure at the base of the skull [42, 43]; the cervical domain comprises neck vertebrae, linking vertebral column and skull; the thoracic domain is characterized by ribs and is followed by lumbar and sacral domains. The caudal domain, post-sacrally, represents the tail in most mammals and the rudimentary coccyx in humans. In birds, the caudal area includes a few small vertebrae and the pygostyle, formed by fused vertebrae [44-46]. Distinctive molecular networks produce these anatomical domains (see section 5, Figure 1C).

In addition to conferring axial identity, Hox genes control the position of limb buds, forming from lateral plate mesoderm. In the forelimb, this is mediated via direct binding to a Tbx5 enhancer element. Co-electroporation of lacZ enhancer reporter plasmids with Hox expression constructs in chick showed that Hox binding sites are required to restrict Tbx5 expression to the forelimb-forming region. This occurs via both activating and repressive interactions. Similar observations were made using mice transgenic for lacZ enhancer reporters. For example, Hoxc9, which is expressed in caudal lateral plate mesoderm, represses the Tbx5 forelimb regulatory element [47]. ChIP-seq experiments revealed overlapping binding sites for Meis, Tbx, and Hox in regulatory sequences of the Fgf10 and Lef1 genes. Thus, these transcription factors converge to co-regulate the Wnt and Fgf pathways, which are critical for limb initiation at the correct axial levels [48]. Comparative analysis of multiple bird species: chicken,

zebra finch and ostrich, with different numbers of cervical vertebrae, suggested that variation in forelimb position correlates with the timing of Hox gene activation during gastrulation [49, 50].

4. Hox regulation of axial identity

Organisation of the body plan requires that local information in the embryo is translated into a functional, global pattern. Hox genes, and axial level-dependent activity of HOX protein combinations, are important in this context.

Amniotes have almost 40 members of the Hox gene family, which are organised into four clusters, arising from duplications of an ancestral cluster, plus subsequent gene loss or duplications (Figure 1A) [2, 51]. Due to these duplications, each gene within a particular cluster has close relatives in one or more of the remaining clusters. These closely related genes share a similar sequence and relative position and are classified into 13 paralogue groups. The position of genes within clusters has functional relevance and reflects the order of gene expression in time and space along the anterior-posterior axis, a feature termed collinearity [52, 53]. Vertebrate Hox clusters are compact in their organisation with uniform orientation of transcription. Genome engineering and inversion of genes within the HoxD cluster in mice showed that this tight structure is likely to be crucial for regulation [54]. Differential genome-wide Hox binding profiles [55], as well as axial level and tissue specific co-factors that facilitate binding to regulatory elements, have been extensively investigated (see for example [56, 57]). Overall, the data suggest that differential binding of Hox TFs drives patterning diversification.

An important question is when axial identity becomes fixed and how this is achieved. Classic transplantation experiments in chick embryos can challenge differentiation potential [40]. Grafting PSM between cervical and thoracic axial levels showed that the dorsal somite retains original Hox expression and morphological identity - specifically rib formation, vertebral and scapula shapes. However, laterally migrating cells are incorporated into ventral derivatives and adopt lateral plate specific Hox expression [58]. Similarly, progenitor cells in the tailbud can adjust their Hox gene

expression after heterochronic transplantation [59]. This suggests that patterns of Hox expression are reversible and extrinsic signals from the environment are able to effect changes to intrinsic features, such as chromatin and epigenetic marks, to influence gene expression programmes.

4.1. Timing of Hox activation

In paraxial mesoderm tissue and in the adjacent neural tube, Hox expression is initiated by temporal activation of Hox genes from 3' to 5' within each cluster. This begins during gastrulation [60] and is also detected in progenitor cell populations located caudally in the embryo [61]. A detailed discussion of these progenitors is found elsewhere in this issue. Importantly, neuromesodermal (NMP) and lateral plate/paraxial mesoderm progenitors (LPMP) together fuel axial elongation throughout embryogenesis. NMPs in particular are specified by high levels of Wnt and FGF signalling in a posterior niche. In the mouse these cells emerge at E7.5, the end of gastrulation, and persist until E13.5 when axis elongation ceases [62]. Lineage tracing in chick showed that limited ingression and increased proliferation maintain and amplify the axial progenitor pool in the tailbud [63], and that these cells contribute to elongation of multiple axial tissues [10]. Transcriptomics, including single cell sequencing, identified their distinct molecular signatures [63, 64]. Their bipotency and eventual differentiation into mesodermal or neural progenies is determined by Brachyury-T and Sox2 transcription factors [61, 65]. Furthermore, analysis of $T^{-/-}$ chimeric embryos suggests that Brachyury-T limits the allocation of primitive streak cells to the NMP pool [66]. Misexpression approaches in chick embryos provided evidence that the Sox2:Brachyury-T ratio affects the cells' motile behaviour: cells stay in place to generate neural tissue (high Sox2) or exit the progenitor zone to produce mesoderm (high Brachyury-T) [67]. Transcriptomics identified a network involving RA and Wnt signalling regulating neural versus mesodermal allocation [68]. The most highly upregulated genes in NMP- and LPMP-containing regions were Hox genes, with peak expression during primitive streak to tail bud transition [64]. The NMP transcriptome changes over time [64] and acquisition of axial identity remains responsive to extrinsic signals [59] until it becomes fixed in the PSM [58]. Interestingly, anterior limits of Hox gene expression

differ in paraxial mesoderm and neural tissues, despite their common origin from posterior progenitor pools [61] and the coupling of axial and paraxial tissue elongation [11].

As these progenitors contribute to more caudal mesodermal or neural progenies, collinear Hox gene activation is translated into a spatial pattern of expression along the axis [69]. The progressive opening of Hox clusters is associated with changes in chromatin structure [70]. In chick this has been visualised using chromatin accessibility and Hox expression within PSM and somites. Differentially accessible chromatin detected across all four Hox clusters reflects the organisation of genes within clusters and their collinear expression. Footprints for transcription factors involved in Hox gene regulation and patterning, such as CDX1/2 and RA receptors, were found in intergenic regions [71].

4.2. Interactions of Hox with lineage specifying genes

Ultimately Hox expression instructs anatomically distinguishable vertebrae subtypes of the vertebral column (Figure 1A, B) [21, 39, 72]. Our understanding of how Hox genes interact with somite patterning pathways to regulate vertebral morphologies is incomplete. For example, cervico-thoracic vertebrae are specified by Hox paralogous groups 5-9 [69]. Hoxa5 is expressed in posterior cervical somites, where Fgf-8 and Shh signalling restricts expression to the lateral sclerotome. Cartilage defects in lateral vertebral elements were observed after Hoxa5 knockdown or misexpression and this is mediated by negative regulation of the chondrogenic transcription factor, Sox-9 [73]. Similarly, Hoxc6 expression demarcates the anterior boundary of the thoracic region in many vertebrates including mouse and chick [74, 75], alligator [76] and snakes [77]. Overexpression of Hox6 paralogues in PSM produces ectopic ribs in more anterior cervical and more posterior lumbar regions, indicating that Hox6 can determine thoracic identity throughout the vertebral column. Mouse knockout studies showed that Hox10 and Hox11 paralogues provide identity to lumbo-sacral regions. In absence of Hox11 function, sacral vertebrae assume lumbar identity, whereas in absence of Hox10 function, ribs project from all posterior vertebrae and no lumbar vertebrae form [78]. Conversely ectopic expression of Hoxa10 suppresses rib formation [79, 80]. Interestingly, both Hox6 and Hox10 interact with an

enhancer element that regulates expression of MYF5 and MRF4/Myf6 in the hypaxial myotome, which in turn activates FGF and PDGF signalling to promote rib formation [80]. Some species, including snakes, have a polymorphism in this enhancer element modulating its response to rib-suppressing and rib-promoting Hox proteins, overall resulting in an expanded rib cage [81]. Ongoing efforts to further characterize downstream gene regulatory networks underlying axial transitions and skeletal morphology use differential transcriptomics and chromatin accessibility in chick somites (SW and AM unpublished). Focussing on cervical and thoracic levels, the aim is to identify molecular signatures that correlate with differential expression of Hox paralogues, which define this region. This may uncover additional target genes and gene networks that mediate axial specific somite patterning and differentiation downstream of Hox.

Studies in conditional mouse mutants have shown that Hox genes are also important in postnatal and adult stages. Lineage tracing showed that Hoxa11 expressing cells are self-renewing skeletal stem cells [82]. Evidence suggests that Hox genes confer differentiation cues to these stem cells throughout life. For example, Hox11 genes play critical roles in skeletal homeostasis of the forelimb. Loss of Hox11 gene function impairs the differentiation to chondrocytes and osteoblasts [83], which fail to mature and normal bone is replaced with an abnormal matrix of collagen fibres [84].

5. Primary and secondary body formation

The vertebrate body can be separated into a primary and secondary body, governed by independent developmental modules acting simultaneously (Figure 1C). The primary body comprises occipital to lumbar domains, the secondary body includes sacral and caudal domains. A recent review describes molecular mechanisms required for each region and the transition between them [85]. Here, we summarise this concept and the regulatory cascades that produce somites with unique signatures.

The primary body arises from epiblast axial progenitors from late gastrulation. Structural and functional differences between axial derivatives are already reflected in somites and mechanisms regulating somite formation vary at different axial levels. This is illustrated by differential effects of

inactivation of Lfng oscillations on thoracic versus caudal vertebrae [86]. Somite maturation dynamics also varies as indicated by the onset of sclerotome and myotome formation [87, 88]. In the occipital domain, the first five somites form occipital bone [42]. They are independent of NMPs, instead, head-associated neural and mesodermal structures require canonical Wnt and Nodal signalling from the primitive streak. This determination of cell fate occurs during gastrulation before morphologically overt segmentation [85, 89].

During transition from occipital to cervical somites, which marks the outset of the spinal cord and vertebral column, a developmental switch occurs and somite development becomes dependent on Brachyury-T and Cdx genes, which promote emergence of NMPs [61]. Cdx2 and Brachyury-T cooperate to establish axial progenitors, loss of function of both genes disrupts axial elongation more than individual mutations [90]. Common targets for Cdx2 and Brachyury-T include Wnt and Fgf pathway components [90, 91]. Wnt3a mediated canonical Wnt signalling becomes important for lineage choice in NMPs, changing their developmental potential to mesoderm during axial elongation [92].

As described above, regional vertebrae characteristics are specified by the actions of Hox paralogues, with clear associations between major transitions in the axial skeleton and anterior expression boundaries of specific Hox paralogues [75]. For most genes, expression persists until chondrification begins in vertebrae primordia. Most vertebrae are specified by a unique combination of Hox genes, thus ensuring formation of discrete morphologies along the axis. For example, in the mouse, the top-most cervical vertebra – the atlas - is characterized by Hoxa1, Hoxa3, Hoxb1, and Hoxd4. The axis, the next vertebra after the atlas, is specified by these four genes plus Hoxa4 and Hoxb4. Segmental identity in the cervical domain is conveyed by Hox paralogous groups 3, 4 and 5, as demonstrated by homeotic transformations in mouse loss-of-function mutants (reviewed in [69]. Axial level specific identity of soft tissues is similarly governed by Hox genes, as shown by regulation of Lbx1 expression in migratory muscle progenitors in limb level somites [93].

A key regulator for the trunk, specifically the thoracic domain, is Oct4. Conditional Oct4 inactivation, produced embryos lacking trunk structures, but still containing tail features [94]. Conversely, sustained

activity of Oct4 produced embryos with extended trunks and delayed secondary body formation. Interestingly, snake embryos have extended Oct4 expression explaining their long trunks [95]. Similarly, Gdf11 signalling is important for the trunk to tail transition, without it the switch is delayed [96], whereas premature activation produces short trunks [97]. Gdf11 also coordinates reallocation of bipotent NMPs from the anterior primitive streak to the tail bud. Single cell transcriptomics comparing primitive streak epithelial cells to tail bud cells, suggests the latter are a subset of epiblast axial progenitors that undergo an incomplete epithelial to mesenchymal transition (EMT). This EMT, triggered by activation of Snai1 downstream of Tgfbr1 and GDF11 signalling, is functionally different from that in the primitive streak and keeps axial progenitors in a transitory state to drive further axial extension, producing post-sacral structures [98]. Tail development relies on the Lin28/let-7 pathway, which controls caudal progenitors by promoting their proliferation and self-renewal, as well as balancing neuromesodermal cell fate decisions [99]. Gdf11-mediated activation of Hox13 leads to premature arrest of posterior axial growth by downregulating Lin28 [100]. In chick embryos, measuring velocities of axis elongation and somite formation shows that activation of posterior Hox paralogous groups 9-13 correlates with slowing down of elongation. The expansion of tail bud axial progenitors ceased and LIN28 was downregulated [101]. Simultaneously, Wnt activity is repressed, reducing mesoderm ingression, this reduces PSM size and brings RA close to the tail bud, causing further loss of axial progenitors. Posterior extension of the axial skeleton is balanced by the actions of the Cdx family of transcription factors, which promote extension, and the Hox12-13 paralogues, which exert a braking effect by regulating Wnt and RA signalling [102]. Together these mechanisms regulate termination of segmentation and axis elongation.

6. In vitro models of somite formation

To dissect coordination of lineage decisions and morphogenetic processes shaping the embryo, a number of sophisticated in vitro systems have recently been developed. The aim of these approaches is to reverse engineer molecular pathways driving embryogenesis. A number of reviews highlight some

key advantages of these model systems [103-108]. They can be grown in large quantities, thus enabling screens, are easier to genetically modify as they are grown directly from ES cells and can be used to study the effect of external signals on morphogenesis. They complement studies with embryos and provide insights into human development that could otherwise not be obtained. Here we summarise findings relevant to axial patterning and somite formation.

As described above, patterning of axial structures depends on the collinear expression of HOX genes in axial stem cells during embryo elongation. It has been unclear whether sequential activation of more posterior HOX genes is controlled by intrinsic chromatin-based timing mechanisms or by changes in environmental signalling cues. A recent study addressed this question using human pluripotent stem cell (PSC) derived axial progenitors, differentiated into spinal cord motor neuron subtypes [109]. Increasing FGF signalling was necessary for progressive activation of caudal HOX genes, consistent with the posterior to anterior gradient of FGF activity in the embryo. Furthermore, exposing progenitors to a combination of FGF and GDF11 accelerated the pace of Hox gene expression and induced more posterior genes and motor neuron subtypes. These results argue that the Hox clock is dynamically controlled by secreted extrinsic signals, although chromatin accessibility or epigenetic modifications were not directly assessed.

Remarkably, three-dimensional aggregates of embryonic stem cells, also termed gastruloids, display germ-layer specification and some axial organization [110-112]. Compared with embryos, gastruloids exhibit limited morphogenesis; this improves dramatically when cultures are supplemented with extracellular matrix. In both mouse and human, single-cell transcriptomics demonstrates that gastruloids activate the same gene networks seen in embryos [113-115]. This includes expression of genes involved in cell-cell adhesion and cell-matrix adhesion, thus allowing the formation of somites. Mouse gastruloids produced striped segmentation patterns and axial elongation reminiscent of those that emerge during somitogenesis. Live imaging confirmed that the somitogenesis clock is active with similar dynamics to in vivo [113]. Furthermore, gene expression analysis revealed lineage segregation of NMPs and their differentiated progeny in an anterior-posterior pattern analogous to that in embryos.

Thus, in these gastruloids, or trunk-like structures, mesodermal and neural differentiation trajectories were coordinated with their position along the axis [113, 115].

Similarly, paraxial mesoderm organoids have been generated from human PSCs [116]. As these somite-like structures (somitoids) recapitulate molecular, morphological and functional features observed in embryos, they could be used to examine development and diseases of the human spine in vitro. There are already several examples of disease modelling using synthetic systems. This includes myasthenia gravis pathology, aspects of which can be recapitulated in organoids containing functional neuromuscular junctions. These neuromuscular organoids were generated from hPSC-derived axial stem cells; both spinal cord neurons and skeletal muscle cells were produced, and their differentiation trajectories were tracked over time by single cell transcriptomics [117]. Chemically defined conditions have also been used to induce multiple musculoskeletal cell types from mouse embryonic stem cells [118] and from human induced pluripotent stem cells (iPSCs) [119]. In the latter, disease modelling of fibrodysplasia ossificans progressiva (FOP), which is characterized by ossification in soft tissues, has further illustrated the usefulness of these protocols [119].

7. Conclusion

Here we summarised mechanisms that generate paraxial mesoderm and reviewed how different anatomical regions of the vertebral column acquire specific identities. A review of pathologies associated with axis development was beyond scope, however, disruptions of the mechanisms described have been shown to cause segmentation defects in human [1, 27]. This spectrum of disorders affect skeletal elements and musculature of the spine, resulting in curvatures such as scoliosis and kyphosis. No doubt, future work on somitogenesis and patterning of the axial skeleton and musculature, in both embryos and gastruloid models, will provide better understanding of the embryonic origin and causes of these diseases. In addition, studies in diverse taxa indicate that changes in HOX expression patterns govern axial identity, suggesting that they are critical for evolutionary adaptations [2, 3]. But how different anatomies are achieved remains poorly understood. Overall the

process of axial regionalisation will continue to fascinate investigators. This is also exemplified by the emergence of ex-vivo systems, such as gastruloids, somitoids and trunk-like structures, where germ-layer specification and axial organization can be dissected in detail.

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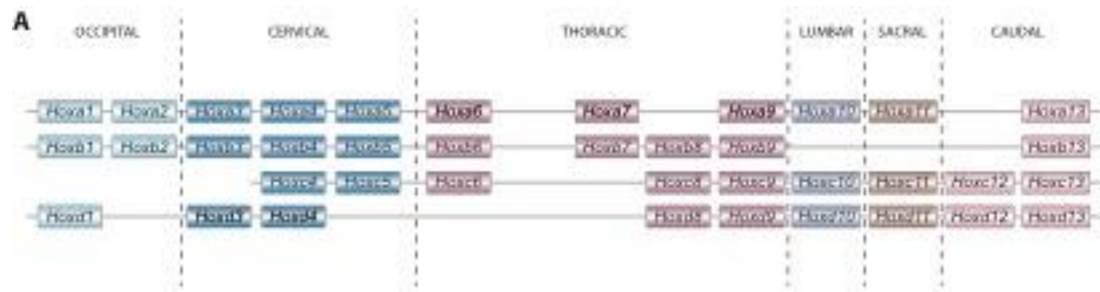
Figure legend

Figure 1: Correspondence of Hox paralogues with vertebral formulae of mouse and chick embryos and primary/secondary body formation. (A) Schematic of the genomic organization of the four Hox clusters (a-d). Hox gene combinatorial expression controls vertebral identity along the anteroposterior axis. Paralogue genes are colour-coded based on their contribution to axial domains (light blue = occipital; dark blue = cervical; dark pink = thoracic; purple = lumbar; brown = sacral; light pink = caudal). Boxes placed perpendicularly depict paralogous relationships within the Hox clusters. **(B)** Schematic representation of the vertebral formula of the mouse compared to the chicken. The somite-derived vertebrae are aligned and subdivided into colour-coded domains corresponding to axial domains, which correlate with specific Hox paralogue expression (A). **(C)** Schematic of primary and secondary body formation and the mechanisms for each. The colour-coded gradient corresponds to the vertebral axial formula in (B) and with specific Hox paralogue expression in (A). The occipital, cervical, thoracic and lumbar domains represent the primary body, whereas the sacral and caudal domains represent the secondary body. PS, primitive streak; NMP, neuromesodermal progenitor.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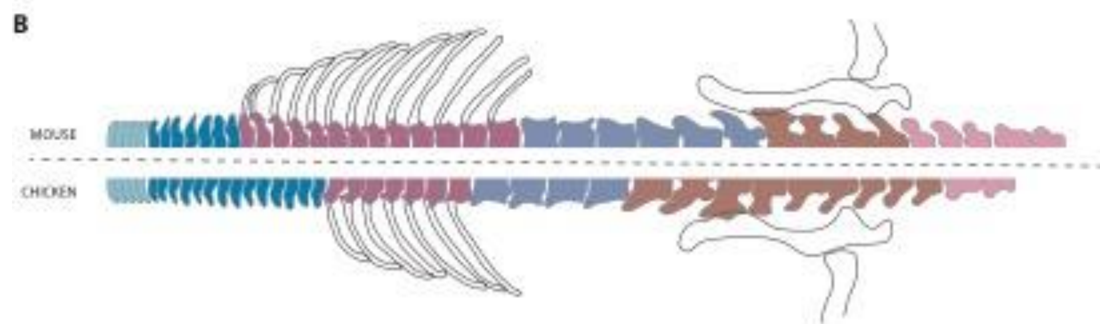
B
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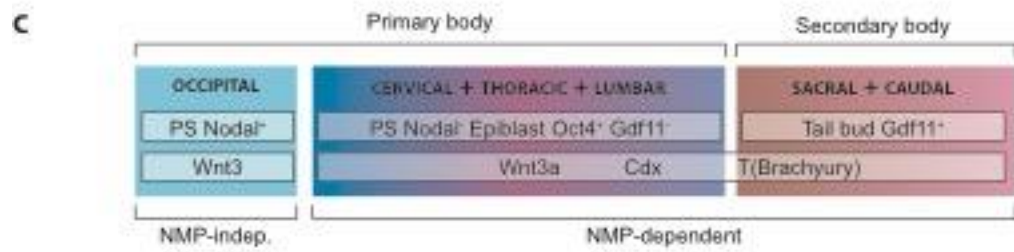
C
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CERVICAL + THORACIC + LUMBAR
PS Nodal^{-} Epiblast Oct4^{+} Gdf11^{-}
Wnt3a
Cdx
SACRAL + CAUDAL
Tail bud Gdf11^{+}
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NMP-indep.
NMP-dependent