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# Building consensus in Neuromesodermal Research: current advances and future biomedical perspectives

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## Abstract

The development of the vertebrate body axis relies on the activity of different populations of axial progenitors, including neuromesodermal progenitors. Currently, the term "Neuromesodermal progenitors" is associated with various definitions. Here, we use distinct terminologies to highlight advances in our understanding of this cell type at both the single cell and population levels. We discuss how these recent insights prompt new opportunities to address a range of biomedical questions spanning cancer metastasis, congenital disorders, cellular metabolism, regenerative medicine, and evolution. Finally, we outline some of the major unanswered questions and propose future directions at the forefront of neuromesodermal research.

## MAIN TEXT

### Introduction

Neuromesodermal progenitors (NMPs), recently reviewed by Wymeersch et al. [1], generate neural (e.g. spinal cord) and mesodermal (e.g. musculoskeletal) derivatives during vertebrate body axis formation. These axial progenitors emerge at the end of gastrulation and reside in the embryo's caudal progenitor region (e.g. tailbud) during axis elongation. Their spatiotemporal localization across vertebrates generally coincides with the co-expression of the transcription factors *Brachyury/(Tbx)T* and *Sox2*. *In vitro*, NMPs can be generated from pluripotent cells and are a critical cellular state to generate spinal cord and musculature. A hallmark of NMPs is their developmental potential/competence, where they can generate cell types from two separate germ layers at post-gastrulation stages. This ability is rare among embryonic cell types. Together with other studies, NMPs have contributed valuable insights that are redefining our understanding of germ layer specification in Developmental Biology [1-5].

Currently, the term 'NMPs' has two different meanings. On the one hand, it has been used to specifically describe individual axial progenitors that give rise to both neural and mesodermal derivatives [6, 7] (hereinafter referred to as NMPs). On the other hand, it has been used to refer to populations of cells harboring NMPs which, as a whole, contribute to neural and mesodermal tissue [1] (hereinafter referred to as Neuromesodermal competent-regions/populations; NMC-region/population). Although displaying a distinct molecular signature from, and not simply a combination of, neural and mesodermal progenitor identities [7, 8, 9], these populations are thought to contain cells with heterogeneous developmental potentials and fates [1,10] (Fig. 1). Crucially, this means that the NMC-region/population is not a mere collection of NMPs (Fig. 1b,c). Hence the need to differentiate between NMPs and the regions that harbor them.

A further distinction can be made between the fate and potency of single cells. This is important because, although the existence of individual cells with the developmental potential to generate neural and mesodermal derivatives (hereinafter referred to as Neuromesodermal competent cells; NMC cells) is probably conserved across vertebrates,

the proportion of individual cells that actually do give rise to both tissues (NMPs) could vary across species [3]. Having distinct terminology will contribute to better phrase the distinction between cells that are in a permissive environment for generating neural and mesodermal descendants, from those that are not. In this review, we use these distinct terms (further highlighted in Box 1) in practice to discuss how NM-research can advance a broad range of discoveries in both basic and translational science (Fig. 2).

### **NM-research, EMT and Cancer/Metastasis**

Epithelial-Mesenchymal Transition (EMT), besides being an important biological process that has its roots in embryonic development, is also a major hallmark of cancer metastasis [11]. Recent studies addressing NMC-populations revealed how Wnt/ $\beta$ -catenin signaling plays an important role in the EMT process that is required to generate mesoderm *in vivo* [1]. For instance, canonical Wnt coordinates a critical developmental checkpoint in zebrafish that ensures coupling of EMT morphogenetic movements with mesoderm cell fate acquisition [12]. In addition to identifying new EMT-markers [13], NM-research sheds new light on the gene regulatory networks (GRNs) that affect EMT processes [14]. Thus, considering that carcinoma formation is reliant on a multistep process comprising the activation of EMT programs, several of them involving Wnt/ $\beta$ -catenin [11], studying the maintenance and differentiation of cells inside the NMC-populations (e.g. NMC cells) opens a new window to identify and modulate, *in vivo*, GRNs and potential drug targets regulating EMT processes with clinical relevance.

The NMC-population might also represent an interesting tool to help clarify the controversial field of epithelial-mesenchymal plasticity (EMP) in cancer metastasis [15]. Transcriptomic analyses in the mouse and live imaging in the chicken embryo have highlighted the dynamic heterogeneity of the NMC-population [7-8, 16–18]. Particularly, single cells within the NMC-region gradually transit from an epithelial to a more mesenchymal state by losing *E-cadherin* and *Epcam* expression while increasing *N-cadherin* and *Vimentin* expression [7-8, 18]. Live imaging experiments in chicken embryos have also highlighted a distinct behavioral signature of NMPs compared to other neighboring mesoderm-fated cells in the primitive streak [7,19]. Together, this suggests that amniote NMC-populations, and particularly NMPs, seem to undergo an incomplete/transient EMT (i.e. EMP) during axis elongation that is different from the one required for mesoderm formation [7-8, 18]. Interestingly, the observed EMP (at least in the mouse embryo) partially depends on *Tgf $\beta$ RI* and resembles metastasis-like EMTs, since the NMC-region in the tail maintains its full developmental potential and self-renewal properties [18, 20]. Overall, this makes the NMC-region/population a potential *in vivo* and genetically tractable system to deconstruct and model the molecular mechanisms underpinning *Tgf $\beta$ RI*-orchestrated metastatic processes and EMP in general.

### **NM-research, Metabolism and Environment**

Metabolomics studies in amniotes have shown that NMC-populations undergo Warburg metabolism or aerobic glycolysis, typical of cancer cells, to regulate body axis elongation [21]. Like cancer cells, embryonic stem cells (ESCs) are dependent on glycolysis to provide sufficient energy to maintain cellular homeostasis and carbon to support cell proliferation and self-renewal. In contrast, differentiated cells have high energy demands,

requiring complete oxidation of metabolic substrates for generating the ATP needed to achieve more specialized tissue functions [22]. Similarly, a gradient of glycolytic activity across the presomitic mesoderm regulates its development and differentiation in the mouse embryo [23], providing a system to dissect how environmental conditions impact cell identity versus self-renewal *in vivo*. In amniotes, the NMC-population has self-renewal properties [7,24] and is closer to a stem-cell-like state than a differentiated one. Indeed, altering glucose homeostasis before tailbud stages induces axial defects [21]. In this case, inhibition of glycolysis phenocopies Wnt/ $\beta$ -catenin signaling inhibition in the tailbud, reducing the number of progenitor cells within the NMC-region that will form the posterior axis [25]. Thus, similar to ESCs and cancer cells, NMC cells could be under tight metabolic regulation to maintain their Warburg metabolic state and self-renewal abilities in amniotes. The metabolic requirements of NMC cells in anamniotes are unknown, raising the question of whether the Warburg metabolic state in progenitor cells is an amniote novelty or an evolutionary-conserved mechanism to form body axes. Importantly, due to their localization in the embryo, NMPs could be exposed to external cues fluctuations. Thanks to recent technical advances, it is now possible to track single cells within the NMC-region in some vertebrate model systems where environmental perturbations are feasible. Moreover, the Warburg-like metabolic state of NMC-populations has been also observed *in vitro* following differentiation protocols from pluripotent stem cells [25]. Thus, combining *in vivo* and *in vitro* methods to understand the role of the environment on the NMC-population homeostasis could bring exciting new insights to a number of fundamental processes. Central to development and disease, is how cells control their identity, and balance the need to undergo differentiation or self-renewal.

## **NM-research and Axial Congenital Disorders**

The NMC-population contributes to both neural and mesodermal tissues during vertebrate axial elongation. Therefore, alterations in the regulation of these cells could result in congenital malformations caused by premature axis termination and aberrant production of neural and/or mesodermal tissues. In humans, such congenital disorders represent a wide spectrum of malformations, ranging from localized defects (e.g. *spina bifida* and posterior neural tube defects (pNTDs)) [26], to axial truncations (e.g. Caudal Regression Syndromes (CRS) [27]. Their etiology is complex and influenced by both genetic and environmental factors affecting a variety of posterior structures. Despite never being directly addressed, the unique roles of the NMC-population, in particular NMPs, during axis extension makes them likely to be affected in the ontogeny of these complex human disorders.

Although the genetic causes of pNTD and CRS in humans remain unknown, similarities between certain mutant phenotypes in mice resembling both pNTDs and some CRS clinical manifestations have a known genetic basis [27,28]. For example mutations in many Planar Cell Polarity (PCP) pathway components, which lead to severe pNTD phenotypes in amniotes [29–31], were also identified in patients with pNTDs [32]. How PCP may impact the induction, maintenance or differentiation of cells within the NMC-region remains an open question. Modulating *PRICKLE-1* in chick embryos [31] or deleting *Vangl2* in cells in the caudal regions of the mouse embryo [33] mimic pNTDs phenotypes, encouraging the investigation of the PCP pathway in NMC-populations. Interestingly, gene association studies in cohorts of patients with congenital axial abnormalities suggest a link between posterior malformations and the *(TBX)T* locus. Individuals carrying one or both

copies of the *(TBX)T*-mutated allele have a 1.6-fold increased risk of *spina bifida* compared with individuals with non mutated alleles [34]. Similarly, homozygous/heterozygous mutations for *(Tbx)T* in mice cause truncations of the caudal region, like CRS, and an aberrant neural tube formation, like *spina bifida* [35,36]. As *(Tbx)T*-expressing cells generate the spinal cord in fish, chick and mouse models [1], and perturbing *(Tbx)T* impacts the maintenance and differentiation of NMPs [8], it raises the question of what role these cells play in human pNTDs.

In combination with genetic variants, environmental/nutritional factors are known to influence correct axial development. For example, high glucose in diabetic mothers and deficiency in maternal folic acid during gestation can lead to conditions affecting axial development [29, 37]. Having the ability to directly manipulate or engineer NMC-populations and study their regulation and lineage differentiation events could provide an opportunity to explore the complex etiology of caudal axial defects, where both genetic and environmental factors play a role.

## **NM-research and Regenerative Medicine**

In addition to improved disease modeling, efforts to engineer NMC-populations *in vitro* from pluripotent cells hold great promise for regenerative medicine and the treatment of spinal cord injuries (SCI). In particular, recent studies suggest that functional recovery in SCI models can be improved when the axial identity of the transplanted neural cells, and the axial position of the injury, is taken into account [38–40]. These findings suggest that neural progenitors derived from different parts of the neuraxis are not equivalent and develop important regional differences that may underpin their functionality. Although the underlying mechanisms require further investigation, designing cell-based therapies for treating SCI would benefit from the engineering of neural cells that resemble a defined region of the spinal cord. While the use of pluripotent cells to generate neural cells *in vitro* has long been considered for the treatment of SCI [41], it is only recently that NMC-populations were engineered *in vitro* and shown to efficiently generate posterior (cervical/thoracic) spinal cord cell types [1].

In addition to implications for spinal cord regeneration [39], reproducing NMC-populations *in vitro* has created many new opportunities to examine features of human development [42,43], and model human disease using induced pluripotent stem cells (iPSCs) [44–46]. In particular, entire neuromuscular junctions can be modeled *in vitro* in 3D from a single NMC-population [44]. These findings highlight the central role that the developmental biology field plays in guiding the differentiation of pluripotent cells to a desired cell type via appropriate intermediate cell states. Combined with advances in high-throughput sequencing, being able to generate NMC-populations *in vitro* now provides an important molecular toolbox to dissect their mechanisms of induction and self-renewal, and explore the variety of cell types they can produce. Quantitative methods such as these can objectively define cell identity based on enhancer usage [47]. These approaches enable the benchmarking of cell types generated *in vitro*, against their *in vivo* counterparts - an essential means to assess cell types generated from pluripotent cells. NMPs are one of the main progenitors of the spinal cord and a potential NMC-region has been documented in humans [48]. Thus, dedicating efforts towards guiding NMC-populations into neural cell types with distinct axial identities holds great promise, both for expanding the range of human diseases that can be modeled using iPSCs, and the development of tailored cell-based therapies for a range of regenerative medicine applications.

## NM-research and Evo-Devo

NMC-regions/populations have been described, to varying extents, in different species. Mouse and chick embryos contain NMPs [6, 7, 19] and other NMC cells [10, 19]. While the zebrafish embryo has been shown, through genetic alterations, to contain NMC cells [49]. A potential NMC-region has also been identified both in *Xenopus* and axolotl [50, 51]. This, together with the observation of a (Tbx)T+/Sox2+ positive domain in the caudal region of these embryos [7, 10, 48, 49, 51], could indicate that NMC-populations, containing NMC cells, are a well conserved vertebrate feature. Future research on the non-vertebrate lineages of cephalochordates and urochordates could further illuminate the distribution of NMC-regions within the chordate phylum. A recent re-appraisal of urochordate embryology is a promising start for this research, as it highlighted that during gastrulation some progenitors give rise to neural and mesodermal cells in the tail. This occurs after segregation of anterior neural and mesodermal lineages [52]. As in many developmental processes, there is broader conservation, beyond chordates, of GRNs involved in axis patterning. A key motif is the *Wnt-(Tbx)T* feedback loop, which is important in NMC-population regulation. This positive feedback is also utilized for posterior axis formation in hemichordates [53] and for oral-aboral axis specification in cnidarians [54,55]. It remains to be studied exactly what might have changed in chordate axis formation to allow NMC cells to arise.

Comparative work can give valuable insight into the regulation of cell behavior in NMC-populations. For example, in contrast to mouse and chick, no NMPs have yet been found in the zebrafish tailbud [56], despite the presence of NMC cells. This is likely due to low cell division levels in the whole tailbud as zebrafish axis extension occurs largely through the rearrangement of cells generated during gastrulation [56]. Comparatively, there are higher levels of cell division in mouse and chick [6, 7]. These variations in developmental modes have been hypothesized to result from variation in maternal nutrient supply [3]. This highlights the importance of considering changes in embryonic growth, timing, and environment between species.

Comparisons of GRNs involved in NMC-populations are also essential for understanding cell behavior. For example, mouse *Hoxb13* is involved in limiting axial progenitor division in the tailbud [57], however the zebrafish orthologue *hoxb13a* is not expressed in the tailbud, in line with little progenitor division in the structure. Instead zebrafish tailbud *hox13* genes have the opposite function - maintaining the progenitor environment [58]. Changes in *Hox13* gene expression has been hypothesized as a contributing factor to variation in amniote tail length [57]. Overall, exploring the variation, across vertebrates, in factors affecting the NMC-population can give insight into posterior body evolution.

## Conclusion

In this review, we propose new terminologies (Box 1) to better integrate and communicate valuable insights from investigations in NM-research at both the single cell and population level. The NMC-region/population represents a heterogeneous group of cells: each cell within it may not possess the same developmental potential or generate the same cell types. Studies utilizing NMC-populations inform our understanding of the collective outcome of a group of cells and how the surrounding environment is important for the fate of single cells within the embryo. This leaves several, key open questions, such as, whether every cell in such a population is equivalent in its ability to generate different lineages, and what the underlying mechanisms determining such differences may be (**Fig. 1c**). The bipotent

nature of NMPs and other NMC cells makes the NMC-region and its equivalent *in vitro* population, an important toolbox to address current challenges in biology and biomedicine (**Fig. 2**). As highlighted in this review, dissecting and modeling EMP in NMC-populations has the potential to provide relevant insights into cancer biology. Understanding the genetic regulation of these cells, and the environmental conditions which impact their behavior, is essential to further our understanding of congenital disease mechanisms and the diversification of posterior body forms across vertebrates. Moreover, being able to derive distinct neural and mesodermal cell types from NMC-populations *in vitro* is important to advance the field of developmental biology and regenerative medicine. All together, discoveries that have emerged from NM-research are a result of multidisciplinary approaches and technologies. Continued collaborative efforts to resolve key questions at the heart of this field (Box 2) may also provide insights with potential clinical relevance.



## Box 1 - Proposed terminology in NM-research

Here, we propose a framework with distinct terminologies that will hopefully help to provide clarity to the existing body of work and to some of the unanswered questions in the field (Box 2). Our framework makes two distinctions. One is between two levels of biological organization: populations and single cells. The other, at the single cell level, is between developmental potential and fate.

The term 'NMPs' was first introduced to describe individual axial progenitors according to the observed neural and mesodermal fate of their progeny in a retrospective lineage tracing analysis [6]. Due to the technical challenges of manipulating single cells, much of what is known about NMPs has been inferred from studies of small regions of the vertebrate embryo or groups of cells derived from ESC differentiation protocols [1]. These populations of progenitor cells were also frequently referred to as NMPs because of their fate and potency, as a whole [1]. However, with an increasing number of studies addressing these populations at both single-cell and population levels [1], such polysemy becomes complicated to discriminate. A distinction between single cells and regions/populations is particularly important, given that studies in the embryo have shown that lineage heterogeneity exists within these regions, revealing that not all cells within a given region or *in vitro*-derived population are NMPs [7, 9, 10, 19, 56, 59].

'Developmental potential' and 'fate' play an instrumental role in developmental biology because they allow researchers to distinguish between what cells are able to do when functionally challenged (their potency) and what they actually give rise to in unperturbed conditions (their fate) [2, 3]. Altering the environment of progenitors either by grafting small regions in the mouse [10] and chick [19], or genetically at the single cell level in zebrafish [49], has been shown to alter the proportion of neural and mesodermal fates compared to normal development. This indicates that although not all cells in these regions normally give rise to both neural and mesoderm (i.e. are not NMPs), some have the developmental potential to generate both tissues. We have termed these cells neuromesodermal competent cells (NMC cells). Making a distinction between NMC cells, which do not give rise to both neural and mesodermal lineages per se, and NMPs, can be particularly useful when comparing cell behavior between different environments, whether it is in different caudal regions of an embryo, different species or different culture conditions.

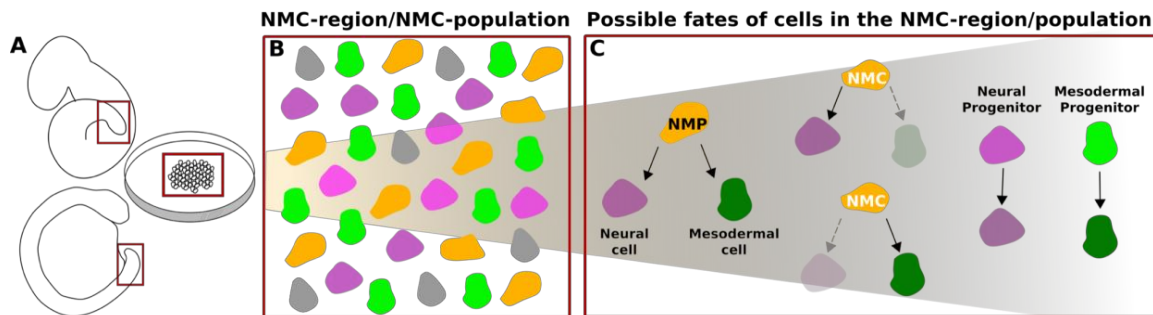
### Proposed terminology

- Neuromesodermal potent/competent cell(s) [NMC cell(s)] - individual cell with the developmental potential to give rise to both neural and mesodermal derivatives during axis elongation.
- Neuromesodermal progenitor(s) [NMP(s)] - individual NMC cell that gives rise to both neural and mesodermal derivatives during axis elongation.
- Neuromesodermal competent region/population(s) [NMC-region(s); NMC-population(s)] - Region/population where NMC cells reside. This bipotent population of axial progenitors contributes to post-occipital neural and mesodermal derivatives. It is generally identified (but not solely defined) by the presence of cells co-expressing (Tbx)T and Sox2.

## **BOX 2 - Open questions in NM-research**

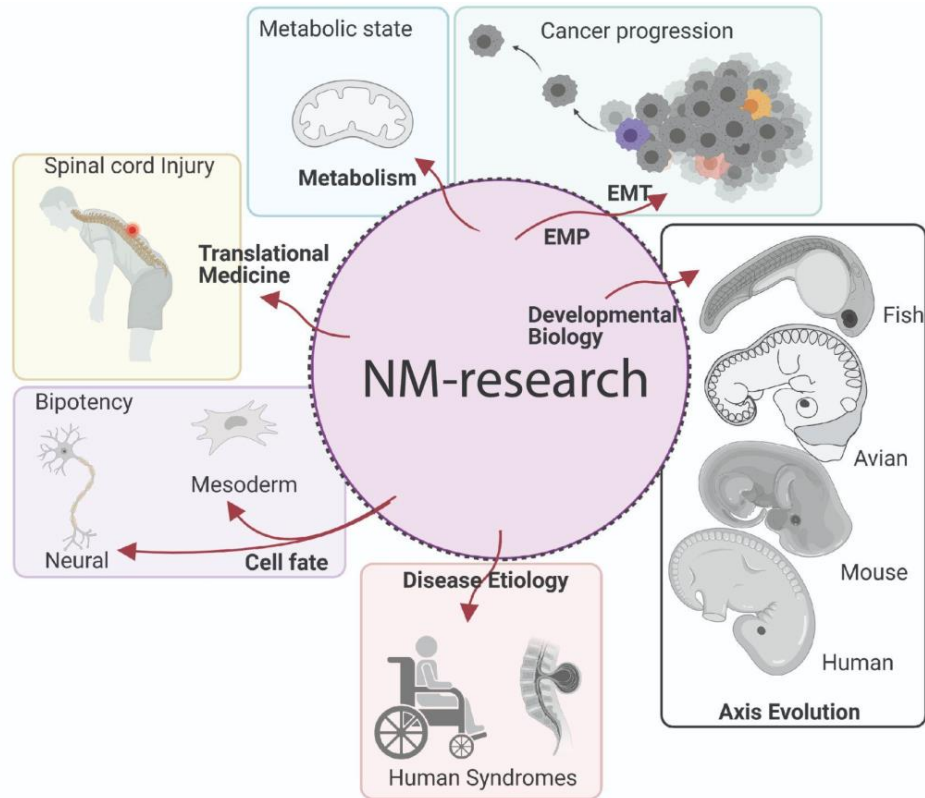
Although cells from NMC-regions in the embryo play an instrumental role in the axial developmental program, our full understanding of their properties and differences both at the single-cell and population level remains unresolved. Below we summarize some of the questions at the forefront of NM research.

- To what extent do NMC-populations and NMC cells (NMPs and non-NMPs), contribute to the body axis?
- What is the proportion/distribution of NMCs and NMPs within the NMC-region? How does this change over time/between species?
- Do NMC cells have a distinct molecular signature from other cells inside the NMC-regions? Does this differ depending on their fate i.e. differ between NMPs and other NMCs?
- What genetic factors and environmental conditions determine the behavior of NMC cells, particularly what determines whether they behave as NMPs?
- To what extent does embryo morphogenesis impact the behavior of NMC-regions across different species?
- How were developmental programs co-opted to give rise to NMC cells and NMPs during evolution?



**Figure 1: The NMC-region/population contains diverse cell types within, which, collectively, contribute to the region/population's fate and developmental potency.**

**A)** Diagram of an amniote/anamniote embryo and *in vitro* differentiation cell cultures with red boxes showing the tail-bud area in the embryo and a group of cells in the dish, where an NMC-region/population is located. **B)** Simplified schematic illustrating that, as a whole, the NMC-region *in vivo* and NMC-population *in vitro*, found in the boxed areas in A, may be composed of: i) neural (pink cell) or mesodermal (green cell) lineage-restricted progenitors; ii) NMC-cells, including NMPs (in orange); iii) other cell-types that may be contained in the region or derived from the *in vitro* differentiation protocols that have not been fully characterized yet (in grey). **C)** Within the NMC-region/population, individual cells can exhibit different potencies and fates. Lineage restricted progenitors (pink and green) will only give rise to cells of either neural or mesodermal lineages, respectively. While some cells contribute to both lineages in unperturbed conditions (NMPs), NMC-cells do not exhibit such 'dual-fated' progeny *per se* and, to date, have only been seen to contribute to both lineages when manipulated by either confronting them to a new environment (by heterotopic grafting) or modifying intrinsic signaling pathways (by genetic inhibition or activation of signaling pathways).



**Figure 2: Illustration of the different fields that can be advanced through NM-research.** Current research in biology and biomedicine can benefit from the study of NMC-regions/populations, NMPs and other NMC cells. Insights from NM-research, both from *in vivo* and *in vitro* studies (pink circle at the center of the figure), provide essential knowledge (red arrows) to advance not only in our understanding of how the body axis is formed during embryonic development across species but also for medical and translational applications in the clinic. EMP: Epithelial mesenchymal plasticity; EMT: Epithelial mesenchymal transition.

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## **Competing interests**

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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## Annotated references: special interest (•) or outstanding interest (••)

- Kinney et al., 2020 Cell Reports (••) - The authors show that, within the NMC-region, mesoderm-fated cells expressing *Sox2* are retained in a partial EMT state. This developmental check-point, also activated by canonical Wnt, prevents the formation of neural cells in the posterior mesodermal regions.

- Tahara et al., 2019 Development (••) - The authors highlight that depletion of *Sall4* in the NMC-population results in a truncated tail. In addition, they also show that this pluripotency-related transcription factor participates in the NMC-population regulation regarding the balance of its neural and mesodermal derivatives.

- Wymeersch et al., 2019 Development (••) - The authors demonstrate that the NMC-regions in the mouse embryo have a molecular signature that changes over time and is distinct from the other axial progenitor populations. Notochord progenitors are required for axis elongation and maintain their transcriptomic identity; therefore, they possibly act as a stable niche for the NMC-population and the NMPs.

- Guibentif et al., 2021 Developmental Cell (••) - The authors use state-of-the-art single-cell technologies to evaluate the dynamics of gene expression of epiblast cells towards the somite lineage, from gastrulation to early somite stage mouse embryo. Their analysis reinforces the non-requirement of *(Tbx)T*, and the NMC-populations, in the formation of the most anterior somites.

- Dias et al., 2020 eLife (••) - The authors analyzed single cells within the mouse NMC-regions and demonstrated for the first time that EMP is at the core of axial extension. Further, they also provide evidence that *Snai1* and *TgfβR1* play a key role in this developmental module required for tailbud formation.

- Guillot et al., 2021 eLife (••) - The authors demonstrate the existence of NMPs in the chicken model using single cell transcriptomics, single cell lineage tracing and in toto live imaging. They found a distinct transcriptional state of the NMC-population, identified transcription factors driving the NMP, Neural and Mesodermal lineages fates, characterized the cell cycle time of NMPs and show that they mature from an epithelial-like state to a mesenchymal-like state.

- Oginuma et al., 2020 Nature (••) - The authors used chicken embryos and human tail bud-like cells differentiated *in vitro* from induced pluripotent stem cells to study the role of glycolysis in regulating fate choices in the NMC-population. They show that modulating glycolysis levels can directly affect the activation of the genes downstream of Wnt signaling to regulate neural and mesodermal fate differentiation.

- Galea et al., 2021, Nat Commun (••) - The authors show that, in the mouse, post-zygotic (*de novo*) deletion of the PCP pathway component *Vangl2*, affecting only small numbers of neuroepithelial cells, is sufficient to cause defects in neural tube closure due to an impairment of apical constriction in a non-autonomous manner. Even though the progenitors of neuroepithelial cells (NMC-population) were not directly addressed, this work provides

evidence that somatic mutations in limited numbers of cells, in a mosaic manner can cause severe caudal axial defects.

- Diaz-Cuadros et al., Nature 2020 (••) - The authors developed *in vitro* protocols to model human and murine muscle differentiation from induced pluripotent stem cells and mouse embryonic stem cells respectively. These protocols involved the generation of the NMC-population during the early steps of differentiation with high efficiency.

- Faustino Martins et al., Cell Stem Cell 2020 (••) - The authors develop an *in vitro* protocol to model human neuromuscular junctions that are generated following prolonged culture of a single NMC-population in 3D. The resulting neuromuscular organoids display central pattern generator-like neuronal circuits that can be used to model neuromuscular diseases such as myasthenia gravis.

- Fritzenwanker et al., 2019 PNAS (••) - The authors demonstrate that *Wnt/(Tbx)T* function in a positive feedback loop in hemichordates. This finding highlights that *Wnt/(Tbx)T* interaction is a conserved part of posterior axis formation in deuterostomes and raises the question of what mechanisms have changed in the chordate lineage for the NMC-population to evolve.

- Lebedeva et al., 2021 Nat. Comms (••) - The authors investigate the regulatory logic of  $\beta$ -catenin dependent oral-aboral axis patterning in the Cnidarian *Nematostella vectensis*. They show that a set of four transcription factor genes, including the *(Tbx)T* orthologue, are in positive feedback loops with  $\beta$ -catenin/Wnt genes and repress more aboral genes to pattern the axis.

## Conflict of Interest

### **Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: