Title: Factors that coordinate mesoderm specification from neuromesodermal progenitors with segmentation during vertebrate axial extension

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Abstract

The formation of the vertebrate body depends on the precise timing and coordination of molecular and morphological events. During vertebrate embryogenesis, the paraxial mesoderm is segmented into structures called somites in a progressive fashion from the anterior to the posterior at the same time as the entire body axis elongates in the posterior direction. Evidence from several vertebrate species indicates that new paraxial mesoderm is continuously induced from neuromesodermal progenitors at the posterior-most end of the embryo. The newly forming mesoderm exists in a specialized environment called the mesodermal progenitor niche. This review will discuss how the progenitor niche coordinates the continuous addition of new mesoderm to the body axis with proper segmentation of this mesoderm upon exit from the niche. I

will focus on evidence that the t-box transcription factor Brachyury and its downstream transcriptional targets serve as the primary factors coordinating mesoderm specification with somitogenesis. I will end with a discussion of recent exciting work regarding the cell-cycle and migratory behavior of mesodermal cells as they exit the progenitor niche, which may serve to further integrate new mesoderm production with proper segmentation.

Keywords: Axial extension, somitogenesis, mesodermal progenitor niche, neuromesodermal progenitors, Brachyury, Wnt

1. Introduction

During vertebrate embryogenesis, the head forms first and the rest of the body forms progressively away from the head. This process leads to a wave of differentiation along the anterior/posterior axis, with anterior cells differentiating first and posterior cells added continuously to the elongating tissues [1]. The progressive differentiation of the body axis is best visualized by observing somitogenesis, the process by which the paraxial mesoderm segments and differentiates into pairs of somites at regular intervals from the anterior to the posterior [2]. The continuous formation of somites requires the sustained contribution of new cells to the paraxial presomitic mesoderm (PSM), which are provided by a population of posteriorly localized progenitors [1]. There is now strong evidence from several vertebrate species that paraxial mesoderm continues to be generated from bipotential neuromesodermal progenitors (NMPs) throughout the entire course of somite formation and axis extension (Fig. 1A, B) [3-9]. During commitment to

the mesodermal lineage from NMPs, cells exist in a niche (also referred to as the mesodermal maturation zone) that has defined molecular and cellular properties (Fig. 1A, B) [4, 10, 11]. This review will focus on how the properties of the mesodermal progenitor niche coordinate both the sustained specification and maintenance of mesodermal progenitors with the proper segmental patterning of the paraxial mesoderm.

2. A brief overview of the clock and wavefront model of somitogenesis

In order to understand how the molecular and cellular properties of the mesodermal progenitor niche affect somitogenesis, it is necessary to provide a basic overview of the molecular mechanism governing this process. For a more in-depth appreciation of the molecular control of somitogenesis, there are some excellent recent reviews [2, 12-14]. In all vertebrates, somites form at regular intervals (the exact timing of which varies between species) in an anterior to posterior progression from unsegmented PSM. As cells leave the anterior PSM and become incorporated into somites, new cells join the posterior PSM as the embryo undergoes posterior axial elongation [14-16]. The clock and wavefront model is used to describe the molecular mechanism of somitogenesis [14-17] (Fig. 1C). The clock represents molecular on-off oscillations of gene expression in somite progenitor cells. Each cell is capable of maintaining oscillating gene expression independently, and neighboring cells become synchronized in their oscillations through the Delta/Notch signaling pathway [18-23]. Many of the cycling genes are themselves members of the Delta/Notch signaling pathway, including the hairy enhancer of split related transcription factors (abbreviated *her* in zebrafish and *Hes* in mouse and chick) and the Notch receptor ligand Delta [14-16]. In mouse and chick but not in zebrafish,

components of the canonical Wnt (hereafter referred to as Wnt) and FGF pathways also cycle in the PSM [24, 25]. In the scope of this review I will focus specifically on components of the Delta/Notch signaling pathway with respect to cycling genes. The conserved importance of Delta/Notch signaling during somitogenesis is reflected in the fact that disruptions in this signaling pathway cause somite defects in all vertebrate embryos in which it has been examined, including mice, chick, *Xenopus*, and zebrafish [16, 26]. Significantly, disruptions in this pathway also cause human vertebral defects leading to congenital scoliosis [16, 27, 28]. The oscillations in gene expression move from the posterior to the anterior PSM at a defined rate (depending on the species), giving the impression of a “wave” of gene expression [15] (Fig. 1). In the anterior-most PSM, the stripe of expression stops oscillating and provides the temporal and spatial information for somite border formation [15]. The wavefront (also called the determination front) is the anterior limit of signaling gradients across the somite progenitor field. The best characterized of these signals are the Wnt and FGF pathways, which are expressed in the posterior-most region of the embryo. As the embryo extends posteriorly, the signaling source and wavefront also move posteriorly. When cells are no longer within a threshold Wnt and FGF activity level, they become competent to respond to the signal generated by the segmentation clock and activate mesoderm posterior genes (*Mesp2* in mouse and *mespb* in zebrafish). The Mesp proteins and their downstream targets orchestrate the physical boundary formation and a new somite forms [29, 30]. An opposing gradient of retinoic acid signaling functions to inhibit Wnt and FGF signaling and helps position the wavefront [12]. As I will discuss further, many of the same signals

and genes involved in the clock and wavefront mechanism of somitogenesis also have essential roles in generating new mesoderm from NMPs and during axial extension.

3. *Brachyury* as a key integrator of mesoderm specification and somitogenesis

Brachyury (abbreviated *Bra*, and also called *T*), which is Latin for “short tail,” was the name given to a mutant line of mice, which exhibited truncated tails in the heterozygous state [31]. The gene responsible for this phenotype was later cloned and identified as the founding member of the t-box transcription factor family, for which there are 17 members in mouse and humans and 26 in zebrafish [32, 33]. *Bra* is expressed, along with the neural marker *Sox2*, in NMPs at the posterior-most end of the embryo in zebrafish, chick, mouse, and humans (as well as in in vitro derived NMPs), and is maintained for a short period in mesodermal progenitors after *Sox2* is repressed [4, 34-38]. In both zebrafish and mouse (zebrafish and other teleost fish have two *bra* genes called *ntla* and *ntlb*, whereas mammals have a single gene), complete loss of *Bra* function results in embryonic lethality where embryos are severely truncated and only form the 1st 8-12 somites (Fig. 2A, B) [39-42]. In all other vertebrates examined, loss of *Bra* function results in axial truncations, including naturally occurring *Bra* mutations, which are a common cause of taillessness in some species of dogs and cats when in the heterozygous state [43-46]. In addition to its striking and well-conserved role in mesoderm specification and axial extension across vertebrate species, *Bra* also appears to be an integral component of the clock and wavefront mechanism of somitogenesis. The 8-12 somites that form in zebrafish and mouse *Bra* loss of function embryos are poorly formed with irregular borders, suggesting a role in proper somitogenesis that is distinct from its

role in mesoderm specification (Fig. 2B) [39, 40]. Consistent with a role in somitogenesis, a human congenital scoliosis study found that a missense mutation in *BRA* is statistically enriched in patients [47]. In the following sections, I will discuss the direct transcriptional targets of Brachyury that facilitate its function in both mesoderm specification from NMPs and somitogenesis.

4. Bra establishes a niche environment of high Wnt and low retinoic acid signaling

Stem or progenitor cell niches in embryonic or adult organisms typically consist of a bipartite cellular arrangement that includes distinct cell types including both support cells and the stem or progenitor cells [48-50]. The support cells can contribute to the physical and molecular properties of the niche such as direct cell adhesion to the progenitors or the secretion of signaling factors that can promote or inhibit differentiation. The mesodermal progenitor population is unique in that the progenitor cells also serve as the support population by creating a signaling environment that can facilitate mesodermal development of cells lacking key mesodermal genes [10].

Bra serves as the key regulator of the molecular signaling environment of the mesodermal progenitor niche. Bra directly activates Wnt ligand transcription in zebrafish, mouse, and human, and Wnt signaling in turn directly regulates *Bra* expression, thereby creating a positive autoregulatory loop [40, 51-53]. Bra also directly activates the transcription of the cytochrome p450 26a1 enzyme (*cyp26a1*), which degrades retinoic acid [10]. Together, these Bra target genes create an environment in the mesodermal progenitors of high Wnt signaling and low retinoic acid signaling. Importantly, in zebrafish, individual cells in the mesodermal progenitor niche that lack the ability to

express *bra* (*ntla* and *ntlb*), Wnt ligands *wnt8a* and *wnt3a*, and *cyp26a1*, can develop normally when surrounded by wild-type mesodermal progenitors, indicating that the progenitors also serve as the support cells. This is in stark contrast to the whole embryo loss of function phenotypes of these genes, where the entire signaling environment of the mesodermal progenitors breaks down and the progenitors are not sustained, leading to truncated embryos with fewer somites than wild-type animals [54-60]. In addition to their role in sustaining mesodermal progenitors, the Wnt and retinoic acid signaling pathways also have critical functions during the segmentation process through regulation of the clock and wavefront mechanism, which will be discussed in more detail below (Fig. 2D).

5. Wnt signaling specifies new mesoderm and affects the clock and wavefront during somitogenesis

Wnt signaling has a dynamic role in both the specification of new PSM and the segmentation process, affecting cell fate and migration in the niche, and both aspects of the clock and wavefront activities during segmentation (Fig. 2D). The posterior localization of Wnt signaling during axial extension is a conserved feature of metazoans [61, 62]. In vertebrates, posteriorly expressed *wnt3a* and *wnt8a* ligands combine to induce new mesoderm, and the absence of these genes causes severe posterior truncations in the number of somites. The relative contributions of *wnt3a* and *wnt8a* to the formation of paraxial mesoderm varies between species, with *wnt8a* playing a greater role in earlier branching vertebrates such as fish and frogs, while *wnt3a* is more important for this process in mammals [40, 55, 56, 59, 63-68]. In all vertebrates, the loss of somites after the loss of Wnt signaling is accompanied by an increase in *sox2* positive neural

progenitors, indicating that Wnt signaling plays a conserved role in the early induction of mesoderm during gastrulation [4, 36, 37, 51, 57, 69-73]. In addition to its critical early role, *wnt3a* and/or *wnt8a* continue to be expressed after gastrulation in the posterior-most embryonic structure called the tailbud, with highest levels in the mesodermal progenitor population. During this sustained posterior expression, Wnt signaling continuously functions to induce new mesoderm from bipotential NMPs in the tailbud, which is recapitulated in NMP cell culture [4, 9, 36, 38, 70, 73]. Together, these studies indicate that Wnt signaling within the mesodermal progenitor niche has an essential function of inducing new mesoderm to join the progenitor pool from NMPs [9].

Wnt signaling promotes mesodermal fate in NMPs through regulation of the t-box transcription factor *Tbx6*. Mammals have a single *Tbx6* gene, whereas zebrafish have three *Tbx6* related genes; *tbx16* (also called *spadetail*), *tbx6* (previously called *tbx24* and *fused somites*), and *tbx6l* (previously called *tbx6*) [32]. In zebrafish, *tbx16* and *tbx6l* are expressed in overlapping domains in the mesodermal progenitors, and *tbx6* is expressed slightly anterior in the PSM [74-76]. Wnt signaling promotes *tbx16* expression, which in turn directly represses *sox2* and establishes the mesodermal fate decision from NMPs [77]. In mouse, Wnt signaling is necessary and sufficient for expression of the single *Tbx6* gene [78], and loss of *Tbx6* function results in an absence of posterior somites and an expansion of neural tissue in regions where somites should normally form [79, 80]. The expansion of neural tissue in *Tbx6* mutants is dependent on the failure to repress *Sox2* in NMPs that exit into the paraxial mesoderm domain [81].

In addition to its pivotal role in mesoderm specification from NMPs, Wnt signaling plays a key role in the normal migration of cells from NMPs to mesodermal

progenitors and finally to PSM. Time-lapse imaging of the zebrafish tailbud showed that NMPs in the dorsal medial zone of the tailbud undergo rapid, coordinated, and directional migration towards the posterior wall of the tailbud [82, 83]. When Wnt signaling is reduced, these cells lose their coherence, causing disruptions in the normal migratory process, which can lead to shortening or bending of the body axis [82, 83]. Further, the Wnt target genes *Tbx6* and the bHLH transcription factor *Msgn1* in mouse, and *tbx16* and *msgn1* in zebrafish, play important roles in the exit of mesodermal progenitors into the PSM. The *tbx16* mutant zebrafish was originally called *spadetail*, due to the fact that cells that normally exit the mesodermal progenitor zone into PSM remain stuck in the tailbud, creating a spade-like appearance [74, 84, 85]. In *spadetail* mutants, anterior somites fail to form, but tailbud exit recovers later in development and tail somites form relatively normally [74, 84, 85]. The exit of cells to form posterior somites in *tbx16* mutants is dependent on *msgn1* function, and in the absence of both *tbx16* and *msgn1* a larger spade forms and all somites are absent [86-88]. In mouse, loss of either *Tbx6* or *Msgn1* function causes a *spadetail*-like phenotype, with mesodermal progenitors failing to exit the niche properly and accumulating in the tailbud [80, 89]. Interestingly, zebrafish *msgn1* mutants form extra somites that are smaller than normal, which is predicted if the somite clock oscillation rate is the same but fewer cells enter the PSM during each oscillation cycle [86]. This is consistent with Wnt signaling controlling the rate of progenitor exit into the PSM [77]. Based on these studies, it is clear that all new mesoderm that joins the PSM throughout axis extension must be induced by high Wnt signaling, providing a unique opportunity for this pathway to pattern the PSM into highly ordered segments.

While Wnt signaling induces new mesoderm and promotes migration and mesodermal progenitor exit into the PSM during axial extension, it also has direct and indirect roles in the molecular clock and wavefront (Fig. 2D). Wnt signaling activity, which is highest in the posterior tailbud domain as it induces new mesoderm, exists in a posterior to anterior gradient [2]. High Wnt signaling levels maintain Notch signaling pathway oscillations in the PSM. Below a threshold Wnt activity, Notch pathway oscillations cease, *mesp* is expressed, and somite formation occurs [90]. In zebrafish, a recent report indicates that Wnt activity ceases posterior to segment formation, indicating that a threshold response of Wnt activity is not directly responsible for initiating segment formation in this species [91]. Instead the Wnt target genes *msgn1*, *tbx6l*, and *tbx16* may transduce the signal for somite formation [91]. *Msgn1* and *Tbx6* are also direct Wnt target genes in mouse, and in addition to their critical roles in Wnt mediated mesoderm specification and exit from the progenitor niche, also have essential roles in somitogenesis. *Tbx6*, which is expressed in the mesodermal progenitors and PSM in mouse, is required, in conjunction with Notch signaling, for the activation of *Mesp2* expression and somite border formation [92]. Although *Tbx6* loss of function mice lack most paraxial mesoderm and therefore also lack most somites [80], a hypomorphic *Tbx6* allele called *rib vertebrae (rb)* forms paraxial mesoderm but fails to segment [93, 94]. Similarly, zebrafish lacking function of the *Tbx6*-related gene *tbx6*, which is expressed in the PSM, form paraxial mesoderm but fail to activate *mespb* and form segments, despite normal cycling of *her1* [76]. On the other hand, mouse *Msgn1* activates the Notch pathway and is required for expression of cycling genes in the PSM [95]. Together, the Wnt target genes *tbx6* and *msgn1*, which induce new mesoderm and promote its exit from

the niche into the PSM, simultaneously help establish both the wavefront and clock during somitogenesis.

6. Cyp26a1 protects the Brachyury/Wnt autoregulatory loop and helps establish the wavefront

Retinoic acid is generated in the posterior-most somites, where the retinoic acid synthesizing enzyme *aldh1a2* is expressed [96, 97]. Despite being in relatively close proximity to the posterior somite retinoic acid source, the mesodermal progenitor niche is an environment of low retinoic acid, achieved through expression of the retinoic acid metabolizing enzyme *cyp26a1* [98-100]. In zebrafish, *cyp26a1* transcription is directly activated by Bra (*ntla*) [10]. The juxtaposition of the RA source and sink creates a linear gradient of RA across the PSM, which can be observed by direct RA visualization [101]. In the absence of *cyp26a1* function in both mouse and zebrafish, RA signaling expands into the posterior-most region of the embryo and causes truncation of the axis with fewer than normal somites forming [54, 60]. This is similar to the axis truncation effect of elevated RA signaling caused by exogenous RA addition [10, 102]. It is essential that mesodermal progenitors exist in a low RA environment because RA signaling is a potent repressor of *bra* (*ntla*) expression, which when repressed causes a breakdown in the Bra/Wnt autoregulatory loop, thus causing a loss of mesoderm specification and creating axis truncations [10, 34]. Therefore, Bra protects its own expression by directly regulating *cyp26a1* expression to prevent RA mediated repression, and simultaneously helps establish a linear RA gradient across the PSM (Fig. 2D).

RA signaling plays an essential role in the positioning of the wavefront during somitogenesis. In addition to its role in repressing Wnt ligand expression, RA signaling represses FGF signaling, including direct repression of *Fgf8* transcription, one of the two ligands (*Fgf4* being the other) involved in establishing the FGF wavefront in mouse [103, 104]. Since the FGF activity gradient in the PSM is established through decay of ligand mRNA, repression of FGF ligand expression directly impinges upon the location of the wavefront [105]. A similar decay mechanism may occur with regard to the Wnt gradient, as both Wnt and FGF ligands are transcribed in the mesodermal progenitors and not in the PSM [2]. Accordingly, loss of RA signaling (leading to an anterior shift of the wavefront) causes smaller somite formation [106]. The situation in zebrafish appears different, as RA positively regulate *fgf8* and *fgf17* expression [107], although like amniotes the absence of RA still results in smaller somites [108, 109].

7. Other direct *Bra* target genes linking mesoderm specification with somitogenesis

Bra is clearly required for the continued generation of new mesoderm across vertebrates, but it also appears to regulate dynamics of the somitogenesis clock, as the 8-12 somites that form after complete loss of *Bra* function in mouse and zebrafish have poorly formed borders (Fig. 2) [39, 40]. While Wnt signaling directly regulates *Bra* expression, and loss of Wnt function also causes a loss of posterior somites, the anterior somites that form in Wnt loss of function embryos have well formed somite boundaries [4, 51, 56, 57, 59]. This is likely due to the fact that while Wnt3a/Wnt8a mediated Wnt signaling directly regulates *Bra* expression, it is required for maintenance of *Bra* expression rather than the initial induction during gastrula stages, which instead is

regulated by Nodal, BMP, and FGF signaling [4, 110-116]. This suggests that Bra regulates somitogenesis at least in part independently of its regulation of Wnt signaling. What Bra target genes might mediate this activity? As previously mentioned, Notch signaling is a critical conserved component of the somitogenesis molecular clock across vertebrate species, and disruptions of the Notch ligand Delta function causes defects in somitogenesis. In zebrafish, Bra (Ntla) directly regulates both *deltaC* and *deltaD* expression [53, 117, 118], and *deltaC* (originally called *beamter*) and *deltaD* (originally called *after eight*) mutants have severe somitogenesis defects [119-121]. Similarly, in mouse, Bra regulates *Dll1* expression, and t-box binding sites in *Dll1* regulatory sequence are essential for its expression [122, 123]. *Dll1* homozygous mutants have severe disruptions in the formation of all somites [124]. Expression of Delta ligands is also dependent on Wnt signaling and the Wnt target gene Tbx6, so loss of expression is likely compounded by the loss of Bra and Wnt signaling in *Bra* mutants, due to breakdown of the Bra/Wnt autoregulatory loop [78, 122].

The Her/HES transcriptional repressors, which serve as core oscillating components of the clock and function within the Notch pathway, are also directly regulated by Bra. In zebrafish, Bra (Ntla) binding was detected in -9kb to +3kb region of both *her1* and *her7* [53], two genes known to be critical for proper somitogenesis [125-127]. *her1* has at least one functional t-box binding site required for transcriptional activation, and *her7* has one of the largest clusters of consensus t-box binding sites of any gene in the zebrafish genome (although untested functionally) [118, 128]. In mouse, *Hes7* is an oscillating gene of the segmentation clock and loss of function results in failure of proper somitogenesis [129, 130]. *Hes7* is a direct BRA target in human and mouse [131].

These results indicate that both the Notch ligand Delta and the hairy enhancer of split family of genes involved in the somitogenesis clock are conserved Bra target genes across vertebrates. Therefore, Bra directly influences somitogenesis independent of its role in mesoderm specification (Fig. 2).

8. Cellular behavior in the progenitor niche – is it noisy in there?

In the clock and wavefront model, individual cells function as independent molecular oscillators. In order for proper somitogenesis to occur, these independent oscillators must be locally entrained to oscillate synchronously, which is accomplished through Delta/Notch signaling [19]. Newly specified mesoderm of the tailbud exhibits behaviors traditionally expected to produce noise in the clock and wavefront model, most notably rapid cell migration and cell division. But recent mathematical modeling and experimental manipulations indicate that these noisy behaviors, to a certain extent, may actually promote molecular clock entrainment rather than inhibit it.

Mathematical models can accurately predict the molecular oscillations that lead to the regular, sequential formation of somites during vertebrate body formation [18, 132, 133]. However, a flaw in many models is the discrepancy between predicted recovery and actual recovery in embryos of molecular oscillations in neighboring cells after transient disruption of cellular synchrony through Delta/Notch signaling inhibition [134]. In mathematical models it would take tens to hundreds of oscillations to recover synchrony, whereas experimental data in zebrafish shows that the embryo can recover synchrony in neighboring cells and proper somite formation within ten oscillations [21, 134]. A revised mathematical model can correct this discrepancy by introducing random

cell movement as cells first begin their oscillations [134]. In the model, the molecular oscillations in neighboring cells, which are synchronized by direct cell-to-cell contact through Delta/Notch signaling, can synchronize rapidly in a group of cells if they increase the number of cells they contact through random cell movement (Fig. 3B) [134]. Random movement and mixing in mesodermal progenitor cells occurs in chick embryos [135]. The random migration in these cells is controlled by FGF signaling, and promotes the axial extension of the developing embryo. In zebrafish, cells in the mesodermal progenitor niche are highly motile, mix readily with each other, and display unsynchronized clock oscillations compared to their neighbor cells. As cells join the posterior PSM, motility and mixing decrease substantially, and oscillatory gene expression appears synchronized between neighbors [20]. Therefore, the rapid and seemingly random mixing of newly specified mesodermal progenitor cells may play a dual role in coordinating axial extension with molecular oscillator entrainment needed for proper somite formation (Fig. 3B). Further experiments are required to determine whether mathematical predications of cell mixing based clock entrainment between neighbor cells occurs in vivo.

Another putative source of noise during the entrainment of molecular oscillators of the somite clock is cell division. In zebrafish, NMPs of the tailbud are mostly held in the G2 stage of the cell cycle, with relatively few mitotic events [136, 137]. As NMPs are induced to form mesoderm and join the mesodermal progenitor pool, they undergo a transit-amplifying step where they turn on the expression of *cdc25a*, a factor that promotes entry into mitosis [136]. The concerted expression of *cdc25a* in cells entering the mesodermal progenitor population causes a synchronized entry into mitosis and a

population doubling [136]. The direct effect of this mitotic event on the cycling of the molecular oscillator of the somite clock was visualized using a transgenic reporter system in zebrafish that allows the direct observation of cycling gene expression, using the *her1* promoter to drive a Her1–Venus fusion protein [138]. Interestingly, the two daughter cells produced after a cell division event have highly entrained cycling reporter expression with each other, even in the absence of Notch signaling where oscillations between neighbor cells tend to be asynchronous. This suggests that equal distribution of molecular components of the oscillator in daughter cells entrains them for future oscillations. Furthermore, cytokinesis occurred predominantly during the off phase of the reporter oscillation, at a time when neighboring cells were also in the off-phase, which minimized the effect of noise between dividing and non-dividing cells [138]. The combined data from these two studies that mesodermal progenitors undergo a synchronous division when they first join the progenitor pool, and that daughter cells have highly entrained clock oscillations, suggests that the initial cell division event shortly after cells acquire a mesodermal identity may actually function to entrain a population of cells, rather than produce noise (Fig. 3C). In support of this model, zebrafish embryos that have a mutation in *emil*, which prevents cells from progressing from G2 to M phase after the gastrula stage, have a greater degree of asynchronous *her1* expression than wild-type embryos at the 15 somite stage, which results in irregular and disorganized somite borders [139]. It will be interesting to see whether other vertebrate species exhibit synchronized mitoses in newly specified mesoderm.

9. Conclusions:

The growing body of literature regarding the properties of progenitor cells in the posterior end of vertebrate embryos has shed light on the deeply integrated processes of continuous mesoderm specification and segmentation, with many of the same genes and signaling pathways mediating both events. The Bra/Wnt autoregulatory loop is a key pathway orchestrating these processes, directly affecting mesoderm specification, cell migration into and out of the mesodermal progenitor niche, and various aspects of both the molecular clock and wavefront during segmentation. It is becoming increasingly clear that many of the factors discussed in this review, in particular Wnt signaling, also integrate other aspects of body patterning. Wnt signaling is involved in regulating Hox gene expression via *caudal homeobox* genes (*cdx*) genes, which impart axial identity (such as whether a segment forms a cervical, thoracic, or lumbar vertebra) to segments [61, 140]. Hox gene expression also controls exit from the mesodermal progenitor niche, and posterior Hox gene expression has recently been shown to negatively regulate Wnt signaling, which subsequently causes posterior axial elongation to cease [141-143]. Thus, the continued investigation of the molecules and cellular properties of the NMPs and mesodermal progenitor niche will shed light on how apparently disparate morphological and patterning events are actually intricately intertwined to globally regulate body plan development during embryogenesis.

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Figure 1 – Mesoderm specification from NMPs is concomitant with somitogenesis.

(A, B) A lateral (A) and ventral (B) view of an embryo illustrating the relative position of cell compartments. Wnt signaling induces new mesoderm from NMPs and promotes exit into the paraxial mesoderm. (C) Four time points are shown during the formation of one somite. Individual cells exhibit oscillating gene expression (indicated by blue color for the “on” phase of the cycle), which are coordinated with neighboring cells. This coordination creates the appearance of stripes of expression, and as they cycle, gives the impression of a traveling wave of gene expression. Cells in the anterior stripe of expression cease their oscillations and remain in the “on” state, and where expression correlates with the signaling wavefront, a new somite border is formed (S1 = most recently formed somite). While this process is occurring, the embryo is extending in the posterior direction. The schematics are from a ventral view with anterior to the top.

Figure 2 – Brachyury and its target genes integrate mesoderm specification with somitogenesis. (A) A wild-type mouse or zebrafish forms 60 or 33 somites respectively. (B) After complete loss of Brachyury in both mouse and zebrafish, the axis is truncated and embryos have only 8-12 poorly formed somites. (C) In vertebrates, Brachyury is expressed in the NMPs and mesodermal progenitors, as well as the notochord (blue). (D) Direct and secondary targets of Brachyury that control mesoderm specification and segment formation (see text for more details of specific target gene function).

Figure 3 –Cell behaviors of newly specified mesoderm hypothesized to coordinate somite clock oscillatory coupling. (A) A schematic showing the transition site from mesodermal progenitor (yellow) to PSM (red). (B) Random cell mixing in chick embryos is controlled by FGF signaling and promotes axial extension. Mixing increases cell contacts, which is predicted in mathematical models to entrain oscillation states of a group of cells through Delta/Notch signaling. (C) In zebrafish embryos, activation of *cdc25a* expression in mesodermal progenitors causes synchronized entry into mitosis, and daughter cells maintain highly entrained oscillatory states with each other. Shades of blue represent oscillatory states of *her/Hes* expression.

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Figure - 2

Figure - 3