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Early Arterial Differentiation and Patterning in the Avian Embryo Model

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Abstract

Of the many models to study vascular biology the avian embryo remains an informative and powerful model system that has provided important insights into endothelial cell recruitment, assembly and remodeling during development of the circulatory system. This review highlights several discoveries in the avian system that show how arterial patterning is regulated using the model of dorsal aortae development along the embryo midline during gastrulation and neurulation. These discoveries were made possible through spatially and temporally controlled gain-of-function experiments that provided direct evidence that BMP signaling plays a pivotal role in vascular recruitment, patterning and remodeling and that Notch-signaling recruits vascular precursor cells to the dorsal aortae. Importantly, BMP ligands are broadly expressed throughout embryos but BMP signaling activation region is spatially defined by precisely regulated expression of BMP antagonists. These discoveries provide insight into how signaling, both positive and negative, regulate vascular patterning. This review also illustrates similarities of early arterial patterning along the embryonic midline in amniotes both avian and mammals including human, evolutionarily specialized from non-amniotes such as fish and frog.

Keywords

angiogenesis; vasculogenesis; chick; Noggin; Chordin

1. Introduction

The avian system is a powerful model system to study vascular biology. The popularity of the avian system is directly related to the accessibility of the vascularized tissue of the embryo proper and extraembryonic membranes. Embryonic vascularized tissues are first observed in the two day old embryo where the many branches of the vascular system can be directly viewed as the blood circulates without rupturing vessels or disturbing embryonic development. *Ex utero* development, as occurs in the amniote egg, makes possible the direct observation of development and experimental intervention at anytime during development by windowing the egg or in shell less embryo cultures (Fig. 1). The accessibility of the embryo in *ex utero* development is a marked advantage over mammalian uterine development which creates an insurmountable barrier to directly viewing normal development outside the uterus without rupturing the vascular system. Non-amniote vertebrates (amphibians and fish) also develop *ex utero* however amphibians and fish

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vascular development shows marked differences in the initial embryonic vessel pattern and recruitment of vascular precursor cells [1-3]. Bird embryos on the other hand show remarkable conservation in embryonic vascular patterning and vessel cell recruitment with mammals[4-7]. Taken together, avian embryos have similar accessibility as the highly amenable fish and frog system while maintaining a high degree of conservation of vascular patterning with human and other mammals for effective vascular modeling.

The experimental avian embryo includes the domestic chicken (*Gallus gallus*) and the Japanese quail (*Cortornix japonicum*). Several modern advances in the study of vascular development in avian embryos includes a sequenced and annotated genome [8] and newly developed methods of transgenesis [9, 10] that have significantly enhanced the existing strengths of the avian system which include excellent vascular markers and antibodies[5, 11, 12], *Gallus in situ* hybridization database (geisha)[13, 14], the ease of embryonic manipulations[15-17], cell lineage analysis[16-18] refined methods of *in vivo* and *in vitro* culture[16, 17, 19] and pseudogenetic misexpression and gene attenuation [9, 10, 20-22]. Most importantly for vascular studies, the diversity of misexpression methods in the avian system makes possible molecular interventions to study how the vascular pattern is shaped by non-autonomous signaling (cell surface and secreted factors expressed by surrounding tissues) and autonomous vascular regulators expressed by endothelial cells. Expression of transgenes for avian vascular studies has been successfully achieved by direct DNA electroporation[11], replication incompetent retrovirus or adenovirus[23, 24], replication competent virus[25], lipofection[26, 27] and misexpression from implanted mammalian cell aggregates [11, 28] although the ability to directly apply peptides provides a simpler method than gene-based misexpression [29-31]. Whereas gene-specific attenuation at the RNA level can be achieved through avian misexpression methods using RNAi or electroporation of modified oligonucleotides (morpholinos) [32, 33], these methods are less effective and reliable as mouse gene deletion technology. The most notable advance in avian vascular biology has been the development of live-embryo videography in conjunction with vascular-specific Tie1-GFP transgenic quail lines to directly observe amniote vascular cell movements[34, 35]. Combining the ability to monitor live movements of endothelial cells with the capacity to experimentally intervene at any moment during vascular development makes the avian system an ideal model to tackle challenging questions of vascular biology through direct evidence.

The avian egg provides two popular assays of vascular development with the vascularized extraembryonic chorioallantoic membrane (CAM) and the emerging and remodeling vessels in the embryo proper[36, 37]. The CAM is a widely utilized vascular assay that has classically been utilized to study paracrine factors that influence vessel growth and patterning. The CAM assay uses a window in the narrow end of the egg shell to expose the, soon to be, vascularized chorioallantoic membrane to intervention via experimental inputs (chemicals, peptides or tumor cells) that are applied to the surface of the vascularizing membrane and the resulting effect on vessel development is observed *in ovo*[36]. As the developing chorioallantoic vessels are elaborations of existing vessels, this assay is strictly angiogenic. However, using the embryo proper a greater number of vascular developmental events can be modeled including both vasculogenic and angiogenic processes[38]. Within the first three days of development in the avian embryo vascular progenitors are specified, emerge and assemble, form vessels and undergo remodeling. In this review we focus on the avian dorsal aortae model where recent studies have discovered the signaling that controls vascular precursor recruitment, control of vessel patterning and remodeling.

2. Three distinct zones of early vascular network differentiation and patterning along the embryo midline

Vascular network development begins with endothelial cell differentiation and capillary plexus formation during gastrulation (Fig. 2A, B). In the amniotes, including birds and mammals, these two initial events of blood vessel formation take place throughout the embryonic disc [4, 39, 40]. There is one exception at a region along the embryonic midline where endothelial cell differentiation, migration, and tube formation are all absent (Fig. 2A-C). Bilateral to this midline negative zone, the first arterial formation takes place and establishes the paired dorsal aortae (Fig. 2C). In addition to these two distinct zones, there is the third zone that continue capillary plexus formation lateral to the paired dorsal aortae. In the head region, the dorsal aortae remain paired throughout development. In the flank region, it is maintained until the bilateral dorsal aortae fuse to form a single dorsal aorta (Fig. 2D-F). Importantly, width of both the midline avascular zone and dorsal aortae is conserved in embryos (Fig. 2C, G). By contrast, position of individual capillaries within the plexus zone vary embryo to embryo (Fig. 2C, G). Thus, the pattern vessels in the vascular plexus is random showing no lateral symmetry and the plexus pattern is unique embryo to embryo. In contrast, both position and size of the first arteries and their remodeling along the embryo midline are precisely defined in the embryo in a temporal and spatial specific manner. This review will discuss the mechanism underlying induction and maintenance of the midline avascular zone and differentiation and remodeling of the dorsal aortae.

3. Modeling vascular patterning using the first embryonic artery system, dorsal aortae

The avian dorsal aortae are an efficient and effective model system to study vessel formation and remodeling. As discussed above, the dorsal aortae or descending aortae first develop as bilateral vessels which are connected to the heart outflows at the anterior end of the embryo and merge with the venous sinuses of the vascular plexus at the posterior end of the embryo (Fig. 2). While the anterior end of the dorsal aortae are, for the most part, static structures the posterior end of the aortae continues to elongate posteriorly as the embryo enlarges. At the second day of development, these lumined bilateral vessels actively transport blood although structurally they are simple endothelial cell lined tubes that are unsheathed by vascular smooth muscle cells. Then the dorsal aortae begin to undergo two forms of remodeling. The first remodeling event is the creation of intersegmental vessels which bridge the dorsal aortae with the posterior cardinal vein through a path that transverses the space between somites. As the intersegmental vessels form, the second major remodeling event commences with the fusion of the dorsal aortae at the midline to form the single dorsal aorta or descending aorta (Fig. 2D-F; Fig. 3). The dorsal aortae fuse along the length of the trunk with the exception of the anterior region of the embryo where the dorsal aortae remain bifurcated to connect with the heart outflows and at the posterior end of the embryo to connect to the umbilical arteries. As aortae fusion occurs the dorsal aorta(e) becomes encircled in vascular smooth muscle derived from the somite and takes on the appearance of a mature artery[41, 42]. Both intersegmental vessel development and dorsal aortae fusion occur in a spatial and temporal restricted manner along the anterioposterior axis with the anterior most region undergoing remodeling first followed by the posterior region[34]. The avian dorsal aortae is an efficient model of vascular patterning as aortae formation occurs by the second day of development and intersegmental vessel formation and aortae fusion occurs shortly after, thus the avian system provides a method to study vessel formation and remodeling in a quarter of the developmental time of a mouse embryo. It is not well understood how all elements of trunk vascular pattern are created but our study has shown

that the positioning of the dorsal aortae and subsequent aortae fusion is created by interplay of positive and negative signals expressed by the axial tissues and the endoderm[11, 28, 43].

4. Sources of positive vascular signals

Positive vascular signals promote vessel formation and are expressed abundantly in the mesoderm and endoderm of the trunk region of the avian embryo[11, 28, 44-47] (Fig. 4A-C). Growth factors with vessel promoting activities during dorsal aortae development include vascular endothelial growth factor (VEGF)[31, 48], members of the fibroblast growth factor (FGF) family[49-52], bone morphogenetic proteins (BMP)[28] and sonic hedgehog Shh[29]. Endothelial cells respond differently to distinct positive vascular factors in avian embryo assays. VEGF has activity to proliferate and attract endothelial cells and increase angioblast recruitment (angioblast are newly formed endothelial cells from mesoderm)[29, 50]. Experiments in avian embryos show that VEGF expands endothelial cell numbers essentially fusing vessels across the embryo proper with the exception of the embryonic midline[11, 25, 31, 45]. FGF has activities to increase vascular cell recruitment from paraxial mesoderm and is believed to play a role in angioblast recruitment[49, 50, 52]. BMP can fuse vessels across the embryo proper, including the midline, and increase vascular cell recruitment from paraxial mesoderm[26, 43]. Shh has activity to increase vascular cell recruitment via a VEGF dependent mechanism [53] and can also induce vascular cell assembly of angioblasts independent of VEGF upregulation[29]. It might be expected that vascular patterning and remodeling events are preceded by the upregulation of specific positive vascular factors, however this is not the case. Expression of positive vascular factors in chick is temporally uniform during major vascular patterning events (embryonic stages 9-15) and does not provide an explanation for initiation of major vascular patterning events in these stages of development. VEGF is broadly expressed in all germ layers with notable enrichment in the somites, notochord, endoderm and lateral mesoderm[11, 28]. Shh is expressed in the notochord at the midline and laterally in the endoderm[29] while BMP2,4,5 and 7 are enriched laterally in all germ layers[11, 28]. The vascular pattern is neither explainable by spatial and temporal regulation of positive vascular factors nor the expression of receptors for positive vascular factors. Our current understanding is that embryonic endothelial cells express receptors for positive vascular factors at similar levels and show the capacity to respond to all positive vascular factors[11, 28, 52, 54]. Through examination of positive vascular signaling alone it is unclear how the dorsal aortae are precisely positioned or how the midline avascular space is created when that region of the embryo expresses necessary vessel promoting factors.

5. Inhibitory vascular signals

The spatial and temporal expression of negative vascular factors provide an explanation for the positioning of the dorsal aortae, remodeling the dorsal aortae during fusion and recruitment of vascular precursor cells (Fig. 3). Vascular inhibitory factors inhibit the formation of vessels by blocking positive vascular signals. During the first three days of vascular development in chick, the best studied vascular inhibitors are Chordin and Noggin which are secreted inhibitors of BMP ligands[11, 28, 43, 55]. The embryo also expresses other vascular inhibitors including Chordin related-1 (Crim1)[56, 57], Follistatins (Fst and Fstl1) [58, 59] and Semaphorin 3 members [60] however only Noggin and Chordin have proven roles in the regulation of vascular patterning in the day 2 to day 4 old chick embryo[11, 28, 43, 55]. There exist several other described vascular inhibitors although no specific role in embryonic endothelial cell recruitment or remodeling has been reported [61-63]. In this review we focus on the well described roles of BMP inhibition by Noggin and Chordin and the role of these negative vascular factors on dorsal aortae development.

6. Positioning the dorsal aortae

Noggin and Chordin expression by the notochord explains the avascular space and positioning of the dorsal aortae in the two day old chick embryo. Noggin and Chordin are expressed in the notochord along the midline at stages of development when the dorsal aortae assemble from angioblasts[28]. Production of vascular inhibitors by the notochord prevents endothelial cells from entering the midline region to form vessels[28,43]. The result of this local inhibition is an avascular zone at the midline with the aortae positioned bilaterally. We propose that the interplay of positive and negative vascular factors is responsible for the sharp proximal border of the dorsal aortae adjacent to the avascular space (Fig. 3). Positive vascular signals VEGF and Shh occur throughout the axial region suggesting that the midline is a favorable position for aortae formation however local expression vascular inhibitors at the midline prevents a midline aorta at the second day of development[11, 28]. Negative vascular factors at the midline presumably redirects aortae formation lateral to the midline over the somite where positive vascular factors are present but distal to sites of negative vascular factor expression (Fig. 3). Thus, positive vascular factors create an environment where the aortae formation is favored at the midline however it is negative vascular factors that position the aortae bilaterally by midline inhibition.

Bilateral aortae and midline repositioning of those aortae to fuse and form a dorsal aorta is an amniote invention. Lower vertebrates (fish and frog) directly recruit endothelial precursor populations from lateral mesoderm to the midline forming the dorsal aorta [2, 3]. The signal to position the fish and frog dorsal aorta precursors occurs by localized expression of positive vascular factors by the hypochord which is an endodermally derived chord-like tissue that lies ventral to the notochord at the embryonic midline [2]. The hypochord directs midline positioning of the dorsal aorta by expressing a substantially higher level of VEGF than the surrounding axial tissue[2]. Thus, non-amniote vertebrates use a simple system of midline positive vascular factor expression to position the dorsal aorta. This simple method of dorsal aorta positioning in nonamniote vertebrates evolved greater complexity in amniotes to provide precise positioning of bilateral aortae. A major modification in amniote embryos is the absence of either a hypochord or midline enriched expression of VEGF[28, 64]. Positioning of bilateral aortae in amniotes is achieved by midline localized negative vascular signals in the presence of broad axial expressed positive signals. Evolutionarily it is unclear if the adaptation of paired aortae in amniotes was necessary for blood circulation or if paired aortae resulted for non-circulatory reasons relating to BMP-antagonist-dependent midline or axial patterning. It is unclear why amniotes adapted bilateral aortae however our studies show how BMP-antagonist expression by the midline regulates aortae formation and positioning.

7. Remodeling of the dorsal aortae

Negative vascular factors signal the remodeling of the bilateral aortae into a single midline vessel under the notochord (Fig. 2 and 3). Aortae fusion initiates after the second day of development and is signaled through a down-regulation of Noggin and Chordin in the notochord in the absence of an increase in VEGF or Shh. Chordin and Noggin are initially expressed throughout the notochord at stage 9 when the aortae are bilateral, however the aortae zipper together from anterior to posterior as inhibitory influence of the notochord decreases[11, 28]. The zippering of the aortae mirror an anterior to posterior decrease in Chordin levels in the notochord[11]. As other BMP-antagonist such as Noggin are no longer expressed by the notochord at the time of fusion Chordin is sufficient to control aortae fusion. With the downregulation of Chordin, the aortae respond to the pre-existing positive vascular factor expression by Shh and VEGF near the midline and fusion of the aortae occurs. From examination of aortae positioning and fusion, a common mechanism is

realized where positive vascular factors are necessary for vascular patterning however it is the interplay of positive and negative vascular factors that provides the control of vascular patterning[11].

8. Negative vascular factors regulation of endothelial cell recruitment to the dorsal aortae

The dorsal aortae recruit angioblasts from two sources; (1) the lateral plate mesoderm; and (2) somite mesoderm. Recruitment of these angioblast populations is dependent on BMP-signaling and regulated by negative vascular factors Chordin and Noggin (Fig. 4). Angioblasts arise spontaneously from the lateral plate mesoderm (splanchnic) [45] and recruitment of these vascular cells is enhanced by FGF, VEGF and BMP[43, 50]. The entire lateral plate mesoderm can give rise to a proportion of angioblast when isolated *in vitro* however in the embryo the axial mesoderm does not give rise to angioblast leading to the emergence of bilateral angioblast that assemble, in part, into the dorsal aortae [43]. Angioblast from axial tissue will only arise if the notochord is removed [43] or if Noggin and Chordin expression is down-regulated[43]. It is not yet known if BMP-signaling acts directly on angioblast specification or angioblast survival and proliferation. While Noggin and Chordin act to restrict angioblast recruitment spatially in the lateral mesoderm, in the somite, Noggin, and possibly Follistatin, serve to restrict angioblast recruitment [55]. The somite provides endothelial and vascular smooth muscle cells to the dorsal aorta(e) after the second day of embryonic development when these vessels actively transport blood [42, 65, 66]. The somite derived angioblasts are first molecularly apparent by the upregulation in the dermomyotome [54]. Noggin and follistatin are expressed complementary to KDR in the dorsal part of the somite (sclerotome) and restrict angioblast recruitment to primarily the dermomyotome[55]. In both the lateral plate mesoderm and the somite, BMP-antagonist spatially restrict where angioblast are recruited despite the presence of positive growth factors.

Non-amniote vertebrates recruit dorsal aorta endothelial cells from lateral mesoderm but not axial tissues, including somites[2, 3]. The shared feature of vascular cell recruitment from lateral mesoderm and not from mesoderm near the midline of amniotes and non-amniote vertebrates alike suggests an evolutionary conserved mechanism midline inhibition of vascular cell recruitment, perhaps regulated by BMP antagonists. Despite the conserved patterns of expression of BMP-antagonist and BMP ligands in axial and lateral tissues respectively between amniotes and non-amniotes, no evidence suggests that BMP positively regulates angioblast recruitment in fish. On the contrary, studies in fish show that BMP-signaling negatively regulates vascular cell recruitment. For example; inhibition of BMP-signaling in lateral mesoderm expands angioblast recruitment [67], morpholino depletion of BMP-antagonist *Crim1* inhibits intesegmental vessels formation [68] and a BMP-specific-inhibiting drug does not inhibit early vascular patterning [69]. Although these studies do not clearly distinguish a role for BMP-signaling in embryonic patterning versus endothelial cell development, these studies do suggest that BMP-dependent vascular signaling is not conserved between fish and amniotes. Frog endothelial cells on the other hand show requirement for BMP to specify blood and some endothelial cell populations however a direct role of BMP on endothelial cells has not been shown in amphibians[70, 71]. It appears that BMP function in vascular cell recruitment is a recent adaptation in amniotes as mammalian and avian studies show an essential and important role for BMP in endothelial cell recruitment, patterning, remodeling and human vascular disease[11, 28, 43, 51, 55, 72-74].

9. Mechanisms of BMP-antagonist inhibition of vessel formation

There exist many possible mechanisms for BMP to regulate vascular patterning. BMP directly influences endothelial cells as demonstrated by studies in cell culture with endothelial cell lines[28, 43]. We have proposed that BMP regulates the capacity of endothelial cells to respond to positive vascular factor signals, where endothelial cells are blind to positive factors such as VEGF when BMP-signaling is inhibited. This model is derived from the observation that BMP-inhibition by Noggin and Chordin inhibits vessel formation in regions of the embryo that abundantly express VEGF and Shh[28]. It is currently unknown if endothelial cells are unable to receive positive vascular factor signals when BMP-signaling is inhibited. Our lab currently searches for the molecular mechanism of BMP-signaling on the reception of positive vascular signals, such as VEGF and FGF, in differentiated endothelial cells. Studies of angioblast recruitment from somite show that BMP-signaling can regulate the expression of the VEGF receptor KDR[43, 55] and provide a mechanism as to how BMP-antagonist act as vascular inhibitors. In this model, BMP inhibition represses vascular differentiation by inhibiting KDR expression resulting in inhibited VEGF-signaling. This mechanism explains how BMP-signaling regulates vascular recruitment however it remains unclear how BMP regulates differentiated endothelial cell behavior in vascular remodeling.

10. Notch activation of somite-derived angioblasts

Although Noggin serves as a negative control for recruitment of somite-derived dorsal aortae precursors it was until recently unknown how recruitment was positively signaled[55]. In an elegant study by Sato et al., (2009) using the avian system it was shown that Notch activation in the somite positively recruits dorsal aortae precursors [75]. Using a combined methods of somatic transgenesis and cell lineage tracing it was shown that Notch directs dorsal somite cells to migrate ventrally to incorporate into the dorsal aortae. Notch-transfection of somite cells is presumably sufficient for transformation into angioblast and all Notch-transfected cells, even dorsal somite which do not normally give rise to many angioblast, migrated to the dorsal aortae. How Notch-activated cells locate the dorsal aortae remains unknown (Fig. 5). VEGF has been shown to be a poor chemoattractant for these cells and since VEGF is broadly expressed it is an unlikely mechanism to guide migration to the dorsal aortae[28, 55]. The authors of this study alternatively show that Eph/Ephrin-signaling guides cells to the aortae through selective repulsion with the dorsal aortae being the least repulsive destination[55]. The data do not exclude that a positive attractive signal from the dorsal aortae guides somite-derived angioblast migration. An exciting concept illustrated in this study is that angioblast can be programmed to seek specific targets. Notch and EphrinB2 direct somite-derived angioblast to the dorsal aortae [66] however, multiple target locations exist of different regions of the somite giving rise to separate embryonic endothelial populations. It remains to be studied what signals guide endothelial targeting and the avian system remains the premier model to answer these important questions in vascular biology. This study showed how dorsal aortae precursor cells are recruited from the somite and accompanies other informative avian studies that identified growth factor signals which increase vascular precursor induction from somite[49-51, 55].

11. Conclusion

Through the use of the avian model of dorsal aortae development many important concepts have been described in vascular biology. Through direct positive evidence a role for negative vascular factors in aortae positioning and remodeling has been demonstrated. It is now clear that positive vascular factors are necessary but not sufficient for vascular patterning. Negative vascular factors like Noggin and Chordin shape the amniote vascular

pattern by restricting where vascular recruitment occurs, where vessels can form and when vessels are remodeled. The avian dorsal aortae model has also shown how Notch can regulate vascular recruitment to a specific vessel. These experiments provide unique insight into vascular cell recruitment and vessel patterning where activities of vascular regulators are directly studied rather than inferring gene function from loss of function.

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Highlights

- Review of the avian vascular model with emphasis on the embryonic dorsal aorta(e) to study vessels cell recruitment, vessel positioning and remodeling.
- Review of recent studies showing how vascular inhibitors are key regulators of vessel positioning and regulate vascular remodeling where positive vascular factor expression (VEGF,FGF and Shh) remains unchanged.
- Models of how BMP-antagonists act as vascular inhibitors to influence vascular cell recruitment and vessel remodeling.

Fig. 1. The avian embryonic vascular model

(A) Windowed egg showing the developing 3 day old embryo. (B) Magnified view of the embryo in (A) showing the embryonic vessels are naturally contrasted against the yellow yolk by the circulation of red blood cells. (C-F) Shell-less culture of chicken embryo in glass dish. (C,D) Day 3 embryo. (E) Near hatching stage chick embryo. (F) Magnified view of shell-less cultured embryos and overlying vascularized chorioallantoic membrane. (G) Filter paper or modified New culture of day 2 embryo. (H) Magnified view of embryo in (G) cultured for 24 hours showing normal vascular development.

Fig. 2. The amniote trunk vascular pattern and remodeling of the dorsal aortae

(A-F) Vascular specific QH1 antibody marking endothelial cells of developing blood vessels from stages 8 through to stage 15 [28, 43]. (G) Probability in the distribution of blood vessels in an area surrounding midline at somite levels 10–12 in 14 somite stage quail embryos (modified from Reese et al., 2004). Digital images of QH1-positive vessels of six superimposed embryos. Bar color grades the presence of QH1-stained vessels in an individual area; Brighter signal represents the highest probability. The bilateral brightest bands lateral to the central dark green band correspond to the dorsal aortae and midline avascular zone, respectively.

Fig. 3. Schematic model of dorsal aortae fusion between stages 10 through stage 15

(A) At the paired dorsal aortae (da) stage the midline region is devoid of vessels and is an avascular space (AVS). The dorsal aortae are bilateral vessels positioned between the avascular space and the vascular plexus (p). (B) The bilateral dorsal aortae begin to remodel during the second day of embryonic development and begin to fuse at the anterior region of the embryo. The initiation of fusion results in a single dorsal aorta (DA) at anterior regions of the embryo and paired dorsal aortae at posterior regions. (C) Fusion of the dorsal aortae results in a midline positioned dorsal aorta (DA).

Fig. 4. Positioning and remodeling of dorsal aortae by inhibitory signals from the notochord
(A) Notochord inhibits emergence of angioblasts (red dots) despite the presence of positive vascular signals (+) from the mesoderm and the endoderm. (B) Angioblast assemble into luminated vessels (red circles) with larger vessels, the dorsal aorta (da), positioned lateral to the midline due to maintained vascular inhibitor expression by the notochord and abundant paraxial expression of positive vascular factors. Midline negative signals explain the medial border of the dorsal aortae however it is unclear if another lateral inhibitory sets the lateral boarder of the dorsal aortae (blue question mark). Smaller plexus vessels assemble lateral to the paraxial tissues possibly due to decreased positive vascular signals in the local environment. (C) Dorsal aorta (DA) formation at the midline is signaled by a decrease in

notochord expressed vascular inhibitors (Noggin and Chordin) while positive vascular factors remain expressed at the same levels. The absence of midline inhibition signals fusion of the dorsal aortae to form the midline dorsal aorta. It is the interplay of positive and negative vascular factors that signals fusion of the dorsal aortae. It is not clear why the lateral border of the dorsal aorta(e) also shift medially during fusion which might be signaled by an unknown lateral inhibitory signal (blue question mark).

A Vascular recruitment from presegmental mesoderm

B Vascular recruitment from somite

Fig. 5. Positive and negative signals which regulate vascular cell recruitment from

(A) presegmental and (B) somite mesoderm. (A) Angioblast (red dots) are recruitment from the presegmental mesoderm and are positively regulated by FGF, BMP and VEGF. The recruitment of angioblast occurs over a broad region of the embryo except for the midline where Noggin and Chordin from the notochord from the mesoderm. Angioblast aggregate to form blood vessels (red circle). Noggin and Chordin have activity to inhibit vessel formation in the presence of positive vascular factors VEGF and Shh. (B) Angioblast recruitment from the somite is positively regulated by FGF, WNT, BMP and VEGF signals. The recruitment of angioblast is negatively regulated by Noggin. Inhibition of BMP-signaling by Noggin blocks the expression of the VEGF receptor (VEGFR2/KDR). Angioblast recruitment and migration to the dorsal aortae is signaled by Notch. It is unclear if BMP and Notch interact to signal angioblast recruitment. EphrineB2, a downstream target of Notch, directs angioblast to migrate to the dorsal aortae (red circle).