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Artery and vein formation: a tug of war between different forces

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How arterial and venous fates are established is largely unknown. In the past, circulatory dynamics were thought to be the exclusive cause of arteries and veins being structurally and functionally distinct; however, growing evidence indicates that an orderly progression of molecular signals controls arterial–venous specification in the developing vertebrate vascular system.

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Introduction

Structural differences between arteries and veins have long been recognized by anatomists. The consensus view was that arteries and veins are distinguished by the direction and pressure of blood flow owing to haemodynamic factors (reviewed in Lawson & Weinstein, 2002). With the discovery of ephrin B2 (*Efnb2*) and the B4 ephrin receptor (*Ephb4*) as markers for arteries and veins, respectively, a new concept has arisen in which the specification of arteries and veins is determined by genetic programmes in the developing embryo before the onset of circulation (Wang *et al*, 1998). Efnb2, which is a transmembrane ligand for the ephrin family, is specifically expressed in endothelial precursors that produce arteries, whereas Ephb4, which is a receptor for Efnb2, is found preferentially in veins. In this review, we summarize recent progress in the characterization of the molecular components involved in arterial–venous fate determination.

Vascular morphogenesis

The vertebrate vasculature is a sophisticated system derived from a complex programme involving sequential genetic and morphological events that drive the formation and specification of blood and lymphatic vasculature.

Angioblasts and pluripotent haematopoietic stem cells are derived from a common mesoderm-derived progenitor cell: the haemangioblast (Vogeli *et al*, 2006). Angioblasts generate vascular endothelial cells, whereas pluripotent haematopoietic cells produce the various blood cells and lymphocytes. In mice, the blood cells are initially generated in the yolk sac at embryonic day 7–7.5 (Moore & Metcalf, 1970). They initially aggregate to form islands of blood cells, which are then sheathed by the endothelial cells to form vessels. In vertebrates, vascular development proceeds through two main stepwise processes: vasculogenesis and angiogenesis (Poole & Coffin, 1989). During vasculogenesis, endothelial precursor cells, in response to local signals such as growth factors and the extracellular matrix, undergo specification, proliferation, migration, differentiation and finally coalescence to form the lining of nascent vessels. The vascular network is then remodelled and refined into arteries, veins and capillaries through angiogenesis.

In early zebrafish embryos, angioblasts originating from the lateral posterior mesoderm migrate to the midline, where they assemble and coalesce to form the aorta (artery) and cardinal vein (Zhong *et al*, 2001). Notably, lineage tracing experiments have revealed that the progeny of each angioblast in the lateral posterior mesoderm can be found in the artery or vein but never both, indicating that the fate of each angioblast has been predetermined in the lateral posterior mesoderm (Zhong *et al*, 2001).

Genes controlling the developmental programmes underlying artery and vein specification have been identified in different vertebrate species. Most are signalling molecules, including ligands and receptors for transmembrane receptor tyrosine kinases, and transcription factors. Among them, the Notch signalling pathway that lies upstream of *Efnb2* has been shown to have a crucial role in arterial–venous specification in both zebrafish and mice (reviewed in Gridley, 2007).

Developmental signals that determine arterial identity

Notch guides arterial fate. Notch, which is an evolutionarily conserved transmembrane receptor, has a well-known function in regulating cell-fate decisions during a range of developmental processes in metazoans (reviewed in Artavanis-Tsakonas *et al*, 1999). Notch signalling occurs through cell-to-cell contact that is mediated by the interactions of Notch receptors and their DSL ligands, which are Delta and Serrate in *Drosophila*, LAG-2 in *Caenorhabditis elegans*, Delta in zebrafish, and Delta-like (Dll) and Jagged (Jag) in mice (reviewed in Bray, 2006). Ligand binding activates Notch by inducing two sequential proteolytic cleavages, which results in the translocation of the intracellular domain of the Notch receptor (NICD) into the nucleus. The NICD binds to CSL transcriptional regulators, which are CBF1 in humans, Suppressor of Hairless (Su(H)) in *Drosophila* and zebrafish,

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LAG-1 in *C. elegans* and Rbpj in mice. As a consequence of binding, co-repressors associated with CSL are released, concomitant with transcriptional activation of downstream target genes.

In zebrafish, *mindbomb* (*mib*) mutants expressing an inactive Notch receptor and embryos microinjected with a dominant-negative form of Su(H) show arterial–venous shunts with a disorganized dorsal aorta and posterior cardinal vein (Lawson *et al*, 2001). These embryos also show an absence, or reduced expression, of arterial markers such as *efnb2a* and *notch5*, while conversely displaying an elevated level of the venous marker fms-related tyrosine kinase 4 (*flt4*) within the dorsal aorta. Similarly, ectopic expression of an activated form of *notch5* in the posterior cardinal vein results in decreased venous *flt4* expression. These results indicate that Notch signalling is necessary for the specification of arterial fate.

One set of downstream target genes regulated by Notch in mammals is the HES (hairy-and-enhancer-of-split) and Hey (hairy-and-enhancer-of-split related) families of transcriptional repressors (reviewed in Iso *et al*, 2003). *Gridlock* (*grl*), which is a zebrafish orthologue of mammalian *Hey2*, is expressed early in the lateral posterior mesoderm, and its expression becomes restricted to the dorsal aorta during later development (Zhong *et al*, 2000). The expression of *grl* can be induced by activation of the *notch1* pathway, and can be blocked by inhibition of *notch* activity (Zhong *et al*, 2001). Consistent with previous findings, embryos treated with different concentrations of *grl* antisense morpholino oligonucleotides show variable arterial defects in the posterior trunk, which are dependent on the expression level of *grl*. A low dose of *grl* antisense morpholino oligonucleotides, which maintains arterial integrity, causes embryos to show decreased expression of the arterial marker *efnb2a* with a concomitant increase in venous *ephb4* expression. Reciprocally, overexpression of *grl* reduces the size of the vein and eliminates *flt4* expression, without affecting the artery. These observations reveal that the *notch–grl* pathway regulates the formation of the dorsal aorta and controls arterial cell fate.

Further support for the idea that Notch signalling is essential for arterial fate decisions has come from studies genetically manipulating the activity of Notch in mice. Mammals have four Notch receptors (Notch 1–4) and five Notch ligands (Dll1, Dll3, Dll4, Jag1 and Jag2) (Gridley, 2007). Mice lacking *Notch1/4*, *Dll4*, *Rbpj*, *Mib* or *Hey1/2* display defects in vascular development, including arterial specification (Duarte *et al*, 2004; Fischer *et al*, 2004; Kokubo *et al*, 2005; Koo *et al*, 2005; Krebs *et al*, 2000, 2004). *Dll4* and *Notch4* are particularly important owing to their specific expression patterns in arterial, but not venous, endothelium in the developing mouse embryo (Shutter *et al*, 2000; Villa *et al*, 2001). *Dll4*-null mutants exhibit disrupted arterial endothelial cell differentiation with decreased *Efnb2* and *Cx37* expression concomitant with increased *Ephb4* expression (Duarte *et al*, 2004). These findings suggest a new role for Notch signalling in suppressing venous cell fate, and therefore promoting arterial differentiation.

Sonic hedgehog and vascular endothelial growth factor control Notch signalling. How is Notch signalling induced? *Sonic hedgehog* (*Shh*) is believed to be a crucial inducer of arterial cell fate. *Shh* encodes a secreted peptide, which is derived from the notochord and floor plate, and is important for neural tube and somite development. Elegant studies in zebrafish have shown that embryos lacking *shh* activity, including the null-mutant *sonic-you* (*syu*) and embryos treated with the inhibitor of *shh* signalling cyclopamine, fail to establish arterial identity in the dorsal aorta, with the loss of arterial maker expression and the gain of venous marker expression (Lawson *et al*, 2002). Conversely, microinjection of messenger RNA encoding *shh* in zebrafish embryos leads to a switch from a venous to an arterial fate in the posterior cardinal vein, indicating that Shh is required for arterial endothelial differentiation (Lawson *et al*, 2002).

Zebrafish embryos lacking *shh* activity also fail to express *vascular endothelial growth factor* (*vegf*) within the somites. Reduction of *vegf* expression in zebrafish embryos by antisense morpholino oligonucleotides results in the loss of arterial fate, whereas overexpression of *vegf* in *shh*-deficient embryos rescues vascular *efnb2a* expression, therefore implicating *vegf* signalling as being important for arterial specification (Lawson *et al*, 2002). Although *vegf* is unable to restore artery identity in mutant embryos that are deficient in *notch* signalling, exogenous induction of *notch* activity rescues *efnb2a* and *notch5* expression in the absence of *vegf*, suggesting that *vegf* lies downstream of *shh*, but upstream of *notch* to induce arterial differentiation.

Consistent with these zebrafish studies, recent observations in mice also confirm the prominent roles of Vegf signalling in promoting arterial differentiation (reviewed in Sato, 2003). Murine Vegfa exists—owing to alternative splicing—as three main homodimeric isoforms: Vegf120, Vegf164 and Vegf188 (reviewed in Ng *et al*, 2006). Besides having different molecular masses, these isoforms can display specific receptor-binding properties. The overexpression of Vegf164 in cardiomyocytes in transgenic mice leads to a proportional increase of *Efnb2*-positive vessels (Visconti *et al*, 2002). Similarly, primary embryonic endothelial cells treated with either Vegf120 or Vegf164 show an increased percentage of *Efnb2*-positive cells without affecting cell proliferation or survival (Mukouyama *et al*, 2002).

The cell-surface receptors for all Vegf isoforms are Vegf receptor 1 (Vegfr1/Flt1), Vegfr2 (KDR/Flk1) and neuropilin 1 (NP-1) (Ng *et al*, 2006). During angiogenesis, Vegfr2 seems to be the main signalling receptor, whereas Vegfr1 acts as a decoy receptor that negatively regulates the activity of Vegfr2 (Rahimi *et al*, 2000). In addition, NP-1 is thought to be a Vegf164 isoform-specific co-receptor that facilitates signalling through Vegfr2. Although *NP-1* has arterial-restricted expression, the ubiquitous expression of Vegfr1 and Vegfr2 in all nascent endothelial cells makes it difficult to further decipher the mechanisms underlying the specific arterial effects of Vegf signalling.

Forkhead box c proteins induce Dll4–Notch. *Forkhead box c (Foxc) 1* and *2*, which are two members of the forkhead family of transcription factors, have been recently shown to control arterial specification by regulating the expression of the Notch ligand *Dll4* (Seo *et al*, 2006). *Foxc1* and *Foxc2* are localized in both endothelium and smooth-muscle cells of the aorta (Kume *et al*, 2001; Seo *et al*, 2006). Mice with targeted inactivation of both *Foxc1* and *Foxc2* develop arterial–venous malformations, which are abnormal fusions of arteries and veins. The defective vessels of these compound homozygous-null mutants show decreased expression of arterial markers (*Dll4*, *Jag1*, *Notch1*, *Notch4*, *Hey2* and *Efnb2*), whereas expression of the venous markers chicken ovalbumin upstream promoter transcription factor II (*COUP-TFII*) and *Ephb4* is not altered, suggesting that the vessels have a vein-like nature. Intriguingly, Foxc proteins can directly bind to a forkhead-binding element in the *Dll4* promoter and stimulate its activity. Together, these findings suggest that *Foxc1* and *Foxc2* act upstream from the *Notch* pathway to induce arterial differentiation.

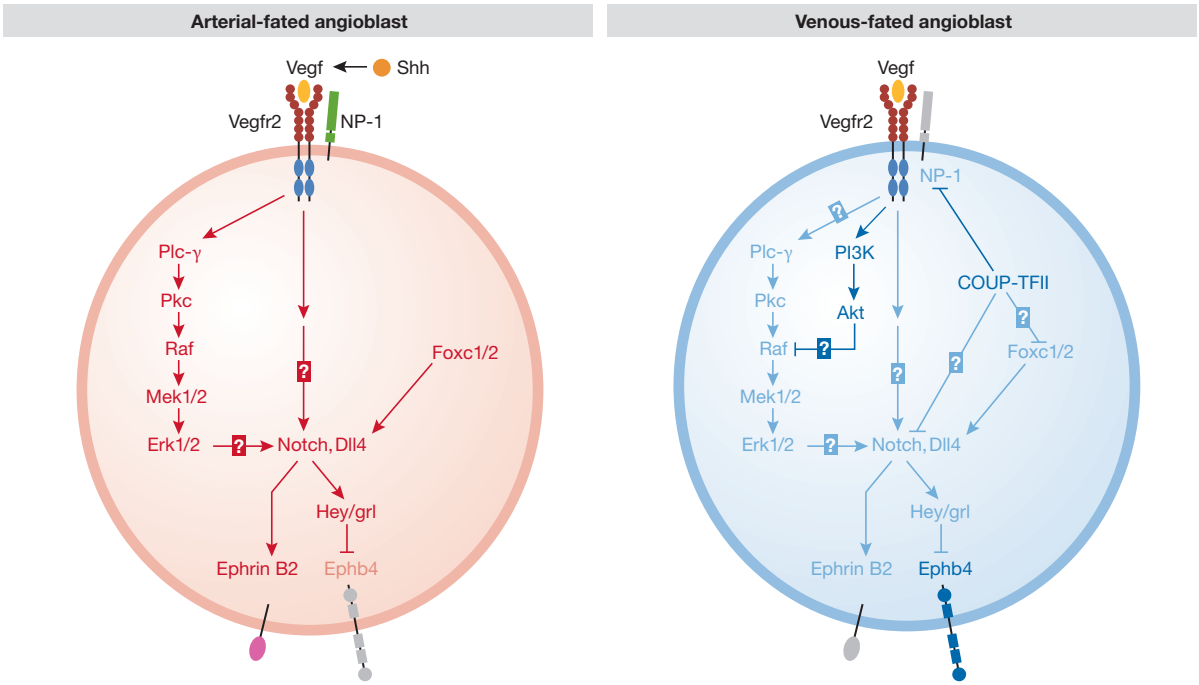

Fig 1 | Model of arterial–venous specification in the developing embryo. Sonic hedgehog (Shh) secreted by the notochord and floor plate induces vascular endothelial growth factor (Vegf) levels in the somites, which in turn activate angioblasts arising from the lateral plate mesoderm. Within an arterial-fated angioblast, Vegf interacts with the Vegf receptor 2 (Vegfr2)–neuropilin 1 (NP-1) complex to activate downstream phospholipase Cγ-1 (Plc-γ)–extracellular signal-regulated kinase (Erk) and Notch signalling pathways, thereby inducing arterial marker expression, such as ephrin B2 (*efnb2*). Forkhead box c 1 (Foxc1)/Foxc2 proteins activate the Notch pathway by inducing the expression of Delta-like 4 (Dll4), thereby leading to an arterial fate. Conversely, chicken ovalbumin upstream promoter transcription factor II (COUP-TFII) and phosphatidylinositol-3 kinase (PI3K)–Akt signalling promote venous fate by suppression of the Notch pathway and Erk signalling, respectively, thereby repressing arterial fate. COUP-TFII not only suppresses the Notch pathway, but also inhibits *NP-1* expression, therefore attenuating Vegf and downstream Notch activation. Unconfirmed interactions are indicated by question marks. This figure was adapted from a model presented by Lamont & Childs (2006). Ephb4, B4 ephrin receptor; grl, gridlock; Hey, hairy-and-enhancer-of-split related; Mek, mitogen-activated protein kinase kinase; Pkc, protein kinase c.

Factors that control venous identity

COUP-TFII marks the veins. The discovery that Vegf–Notch signalling is required for arterial cell specification in the zebrafish led to the belief that the venous state is derived from a default pathway, whereas arterial identity is conferred by the presence of additional signalling (Thurston & Yancopoulos, 2001). This concept was later reassessed by a breakthrough in understanding of the venous functions of COUP-TFII. *COUP-TFII*, which is a member of the orphan nuclear receptor superfamily, is specifically expressed in venous, but not arterial, endothelium (You *et al*, 2005). Conditional ablation of *COUP-TFII* in the endothelium results in the acquisition of arterial characteristics in veins. This phenotype is characterized by an increase in expression of arterial markers, including *efnb2*, *NP-1* and *Notch* signalling molecules. In addition, the mutant veins are able to form haematopoietic cell clusters and recruit smooth-muscle cells, which are two functional features of arteries, indicating that the mutant veins not only gain arterial-specific gene expression but also behave like arteries. Intriguingly, ectopic expression of *COUP-TFII* in the endothelium results in a fusion of arteries and veins, which phenocopies the vascular defects found in mouse embryos lacking *NP-1* or *Notch1*, suggesting that downregulation of *Notch* signalling could account for such vascular phenotypes (Huppert *et al*, 2000; Kawasaki *et al*, 1999). Indeed, the ectopic expression of *COUP-TFII* results in a loss of arterial markers, including *NP-1*, and other factors in the *Notch* signalling pathway. Together, these findings suggest that COUP-TFII is a crucial regulator of venous fate determination.

Phosphatidylinositol-3 kinase also marks the veins. The phosphatidylinositol-3 kinase (PI3K) signalling pathway has also been implicated in the specification of vein identity. Recent studies in zebrafish have identified this pathway as being important for the maintenance of vein identity. PI3K promotes venous cell fate by blocking arterial p42/44 mitogen-activated protein kinase (Mapk; extracellular signal-regulated kinase (Erk)) activation (Hong *et al*, 2006). Activated Erks are preferentially detected in angioblasts that are fated to become arteries. The identification of phospholipase Cγ-1 (Plc-γ) as a downstream mediator of Vegf signalling in the arterial fate decision links Vegf signalling to Erk activation, thereby forming the molecular cascade Vegf→Plc-γ→Pkc→Raf→Mek→Erk (Pkc for protein kinase c; Mek for mitogen-activated protein kinase kinase) (Lawson *et al*, 2003). The flavone GS4898 was isolated using a powerful small-molecule screen, and characterized as an inhibitor of the PI3K–Akt pathway. The inactivation of Akt with GS4898 reverses the inhibitory effects of PI3K on Plc-γ–Erk signalling, thereby triggering an arterial fate specification (Hong *et al*, 2006). Conversely, the mosaic expression of constitutive, active Akt was found to induce venous fate. Together,

these studies suggest a new role for the interplay of the Plc-γ–Erk and PI3K–Akt pathways in controlling artery and vein decisions.

Collectively, the accumulated data from the zebrafish studies delineate a genetic hierarchy of signalling pathways that is responsible for arterial specification (Fig 1). Expression of *Shh* in the notochord and floor plate induces *Vegf* expression in the adjacent somites. The interaction of Vegf with its receptor on angioblasts triggers activation signals that are transduced through the Plc-γ–Erk and Notch pathways, which in turn induce *efnb2* and other arterial markers to establish an arterial fate. Within an angioblast fated to become a venous cell, *COUP-TFII* represses the Notch signalling pathway by inhibiting the expression of *NP-1* and other participants, thereby suppressing an arterial fate (Fig 1).

Conclusions and perspectives

We have highlighted the recent progress made in understanding artery and vein identity. According to the current model, the concept of artery and vein specification works like the ancient Chinese philosophical concept of yin-yang, which describes a balance of two dynamic, opposing and complementary principles in the universe. The outer circle of the yin-yang symbol reveals everything or, in this case, the whole population of angioblasts, whereas the black and white parts within the circle represent two natural forces or, here, two cell fates. This suggests that arterial and venous fate cannot exist without each other under normal conditions, and that an intrinsic relationship between them coordinates harmony.

Although we have discussed at length the fact that vessel identity is genetically predetermined, there is no doubt that haemodynamic factors, such as blood pressure and flow, also have a crucial role in regulating vascular plasticity (reviewed in Jones *et al*, 2006). Quail–chicken graft assays show that flow is able to change the identity of nascent endothelial cells, highlighting the importance of the microenvironment (le Noble *et al*, 2004; Moyon *et al*, 2001). Understanding the balance between global genetic inputs and local environmental forces in vessel identity and remodelling will provide insight into how a functional vascular architecture forms.

Many of the molecular regulators responsible for the arterial and venous fate specification have now begun to be identified, but their roles are still not fully understood. In the case of *COUP-TFII*, some questions still remain; for example, does *COUP-TFII* act in angioblasts? If angioblasts in the lateral posterior mesoderm are restricted to an arterial or venous lineage, does *COUP-TFII* regulate the venous differentiation programme within a subset of angioblasts while the remaining cells take on an arterial fate? Alternatively, if all angioblasts are *COUP-TFII*-positive, could inactivation of *COUP-TFII* by an unknown signal during angioblast migration confer arterial identity? It is equally possible that *COUP-TFII* acts even higher in the hierarchy, for example in the haemangioblast, to regulate cell fate. Other interesting questions that remain include whether PI3K activation is linked to COUP-TFII-mediated signalling, whether Foxc proteins regulate the Plc-γ–Erk cascade downstream of Vegfr2 and, finally, what is the signal/factor regulating the expression of *COUP-TFII* specifically in the endothelium of the vein? We believe that *COUP-TFII* might directly suppress the expression of many participants, such as *NP-1*, *Hey*, *Foxc* and *Notch*, which lie upstream and downstream of the Notch signalling pathway or within the Notch pathway, to ensure that Notch signalling is inactivated in this process. The precise mechanism by which these complicated hierarchies of signalling interactions are coordinated and the interplays between them remain to be elucidated.

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REFERENCES

Artavanis-Tsakonas S, Rand MD, Lake RJ (1999) Notch signaling: cell fate control and signal integration in development. *Science* **284:** 770–776

Bray SJ (2006) Notch signalling: a simple pathway becomes complex. *Nat Rev Mol Cell Biol* **7:** 678–689

Duarte A, Hirashima M, Benedito R, Trindade A, Diniz P, Bekman E, Costa L, Henrique D, Rossant J (2004) Dosage-sensitive requirement for mouse Dll4 in artery development. *Genes Dev* **18:** 2474–2478

Fischer A, Schumacher N, Maier M, Sendtner M, Gessler M (2004) The Notch target genes *Hey1* and *Hey2* are required for embryonic vascular development. *Genes Dev* **18:** 901–911

Gridley T (2007) Notch signaling in vascular development and physiology. *Development* **134:** 2709–2718

Hong CC, Peterson QP, Hong JY, Peterson RT (2006) Artery/vein specification is governed by opposing phosphatidylinositol-3 kinase and MAP kinase/ERK signaling. *Curr Biol* **16:** 1366–1372

Huppert SS, Le A, Schroeter EH, Mumm JS, Saxena MT, Milner LA, Kopan R (2000) Embryonic lethality in mice homozygous for a processing-deficient allele of Notch1. *Nature* **405:** 966–970

Iso T, Kedes L, Hamamori Y (2003) HES and HERP families: multiple effectors of the Notch signaling pathway. *J Cell Physiol* **194:** 237–255

Jones EA, le Noble F, Eichmann A (2006) What determines blood vessel structure? Genetic prespecification vs. hemodynamics. *Physiology* **21:** 388–395

Kawasaki T, Kitsukawa T, Bekku Y, Matsuda Y, Sanbo M, Yagi T, Fujisawa H (1999) A requirement for neuropilin-1 in embryonic vessel formation. *Development* **126:** 4895–4902

Kokubo H, Miyagawa-Tomita S, Nakazawa M, Saga Y, Johnson RL (2005) Mouse *hesr1* and *hesr2* genes are redundantly required to mediate Notch signaling in the developing cardiovascular system. *Dev Biol* **278:** 301–309

Koo BK *et al* (2005) Mind bomb 1 is essential for generating functional Notch ligands to activate Notch. *Development* **132:** 3459–3470

Krebs LT *et al* (2000) Notch signaling is essential for vascular morphogenesis in mice. *Genes Dev* **14:** 1343–1352

Krebs LT, Shutter JR, Tanigaki K, Honjo T, Stark KL, Gridley T (2004) Haploinsufficient lethality and formation of arteriovenous malformations in Notch pathway mutants. *Genes Dev* **18:** 2469–2473

Kume T, Jiang H, Topczewska JM, Hogan BL (2001) The murine winged helix transcription factors, Foxc1 and Foxc2, are both required for cardiovascular development and somitogenesis. *Genes Dev* **15:** 2470–2482

Lamont RE, Childs S (2006) MAPping out arteries and veins. *Sci STKE* **355:** pe39

Lawson ND, Weinstein BM (2002) Arteries and veins: making a difference with zebrafish. *Nat Rev Genet* **3:** 674–682

Lawson ND, Scheer N, Pham VN, Kim CH, Chitnis AB, Campos-Ortega JA, Weinstein BM (2001) Notch signaling is required for arterial–venous differentiation during embryonic vascular development. *Development* **128:** 3675–3683

Lawson ND, Vogel AM, Weinstein BM (2002) Sonic hedgehog and vascular endothelial growth factor act upstream of the Notch pathway during arterial endothelial differentiation. *Dev Cell* **3:** 127–136

Lawson ND, Mugford JW, Diamond BA, Weinstein BM (2003) Phospholipase C gamma-1 is required downstream of vascular endothelial growth factor during arterial development. *Genes Dev* **17:** 1346–1351

le Noble F, Moyon D, Pardanaud L, Yuan L, Djonov V, Matthijsen R, Breant C, Fleury V, Eichmann A (2004) Flow regulates arterial–venous differentiation in the chick embryo yolk sac. *Development* **131:** 361–375

Moore MA, Metcalf D (1970) Ontogeny of the haemopoietic system: yolk sac origin of *in vivo* and *in vitro* colony forming cells in the developing mouse embryo. *Br J Haematol* **18:** 279–296

Moyon D, Pardanaud L, Yuan L, Breant C, Eichmann A (2001) Plasticity of endothelial cells during arterial–venous differentiation in the avian embryo. *Development* **128:** 3359–3370

Mukouyama YS, Shin D, Britsch S, Taniguchi M, Anderson DJ (2002) Sensory nerves determine the pattern of arterial differentiation and blood vessel branching in the skin. *Cell* **109:** 693–705

Ng YS, Krilleke D, Shima DT (2006) VEGF function in vascular pathogenesis. *Exp Cell Res* **312:** 527–537

Poole TJ, Coffin JD (1989) Vasculogenesis and angiogenesis: two distinct morphogenetic mechanisms establish embryonic vascular pattern. *J Exp Zool* **251:** 224–231

Rahimi N, Dayanir V, Lashkari K (2000) Receptor chimeras indicate that the vascular endothelial growth factor receptor-1 (VEGFR-1) modulates mitogenic activity of VEGFR-2 in endothelial cells. *J Biol Chem* **275:** 16986–16992

Sato TN (2003) Vascular development: molecular logic for defining arteries and veins. *Curr Opin Hematol* **10:** 131–135

Seo S, Fujita H, Nakano A, Kang M, Duarte A, Kume T (2006) The forkhead transcription factors, Foxc1 and Foxc2, are required for arterial specification and lymphatic sprouting during vascular development. *Dev Biol* **294:** 458–470

Shutter JR, Scully S, Fan W, Richards WG, Kitajewski J, Deblandre GA, Kintner CR, Stark KL (2000) Dll4, a novel Notch ligand expressed in arterial endothelium. *Genes Dev* **14:** 1313–1318

Thurston G, Yancopoulos GD (2001) Gridlock in the blood. *Nature* **414:** 163–164

Villa N, Walker L, Lindsell CE, Gasson J, Iruela-Arispe ML, Weinmaster G (2001) Vascular expression of Notch pathway receptors and ligands is restricted to arterial vessels. *Mech Dev* **108:** 161–164

Visconti RP, Richardson CD, Sato TN (2002) Orchestration of angiogenesis and arteriovenous contribution by angiopoietins and vascular endothelial growth factor (VEGF). *Proc Natl Acad Sci USA* **99:** 8219–8224

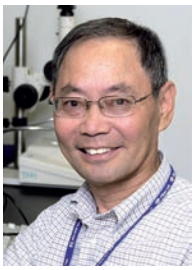
Vogeli KM, Jin SW, Martin GR, Stainier DY (2006) A common progenitor for haematopoietic and endothelial lineages in the zebrafish gastrula. *Nature* **443:** 337–339

Wang HU, Chen ZF, Anderson DJ (1998) Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. *Cell* **93:** 741–753

You LR, Lin FJ, Lee CT, DeMayo FJ, Tsai MJ, Tsai SY (2005) Suppression of Notch signalling by the COUP-TFII transcription factor regulates vein identity. *Nature* **435:** 98–104

Zhong TP, Rosenberg M, Mohideen MA, Weinstein B, Fishman MC (2000) Gridlock, an *HLH* gene required for assembly of the aorta in zebrafish. *Science* **287:** 1820–1824

Zhong TP, Childs S, Leu JP, Fishman MC (2001) Gridlock signalling pathway fashions the first embryonic artery. *Nature* **414:** 216–220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