Molecular regulation of angiogenesis and lymphangiogenesis

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Abstract | Blood vessels and lymphatic vessels form extensive networks that are essential for the transport of fluids, gases, macromolecules and cells within the large and complex bodies of vertebrates. Both of these vascular structures are lined with endothelial cells that integrate functionally into different organs, acquire tissue-specific specialization and retain plasticity; thereby, they permit growth during tissue repair or in disease settings. The angiogenic growth of blood vessels and lymphatic vessels coordinates several biological processes such as cell proliferation, guided migration, differentiation and cell–cell communication.

Angioblasts
Mesoderm-derived endothelial precursor cells that are not fully differentiated and retain some stem-cell properties.

The complex body architecture of vertebrates requires efficient and simultaneous transport of gases, liquids, nutrients, signalling molecules and circulating cells between tissues and organs. This task is carried out by two highly branched, tree-like tubular networks: the blood vessels and the lymphatic vessels, which are formed by endothelial cells (ECs) (FIG. 1). These two endothelial structures are interconnected through the largest lymphatic vessel, the thoracic duct, which drains lymph into the blood circulation. Both endothelial networks are essential for homeostasis in the healthy organism and their malformation or dysfunction contributes to the pathogenesis of many diseases[1–4]. Insufficient blood-vessel supply causes tissue ischaemia in cardiovascular disease, whereas in cancer, stimulation of angiogenesis can give tumour cells better access to nutrients and oxygen. Cancer cells also hijack the lymphatic vasculature or blood vessels during metastasis to distant tissues[2,5,6]. Therefore, the ability to control the growth of blood and lymphatic vessels directly should have considerable therapeutic benefits.

The angiogenic growth of the two vascular systems provides an excellent example for the tight coordination of cell proliferation, differentiation, migration, matrix adhesion and cell–cell signalling processes during tissue morphogenesis. In this review, we provide a broad overview of the known angiogenic and lymphangiogenic regulators, outline their biological roles and highlight open questions that concern the angiogenic growth programme. Although we mainly focus on the regulation of angiogenesis during development, some of the findings are relevant to human diseases in which the normal growth and differentiation of blood vessels or lymphatic vessels are compromised.

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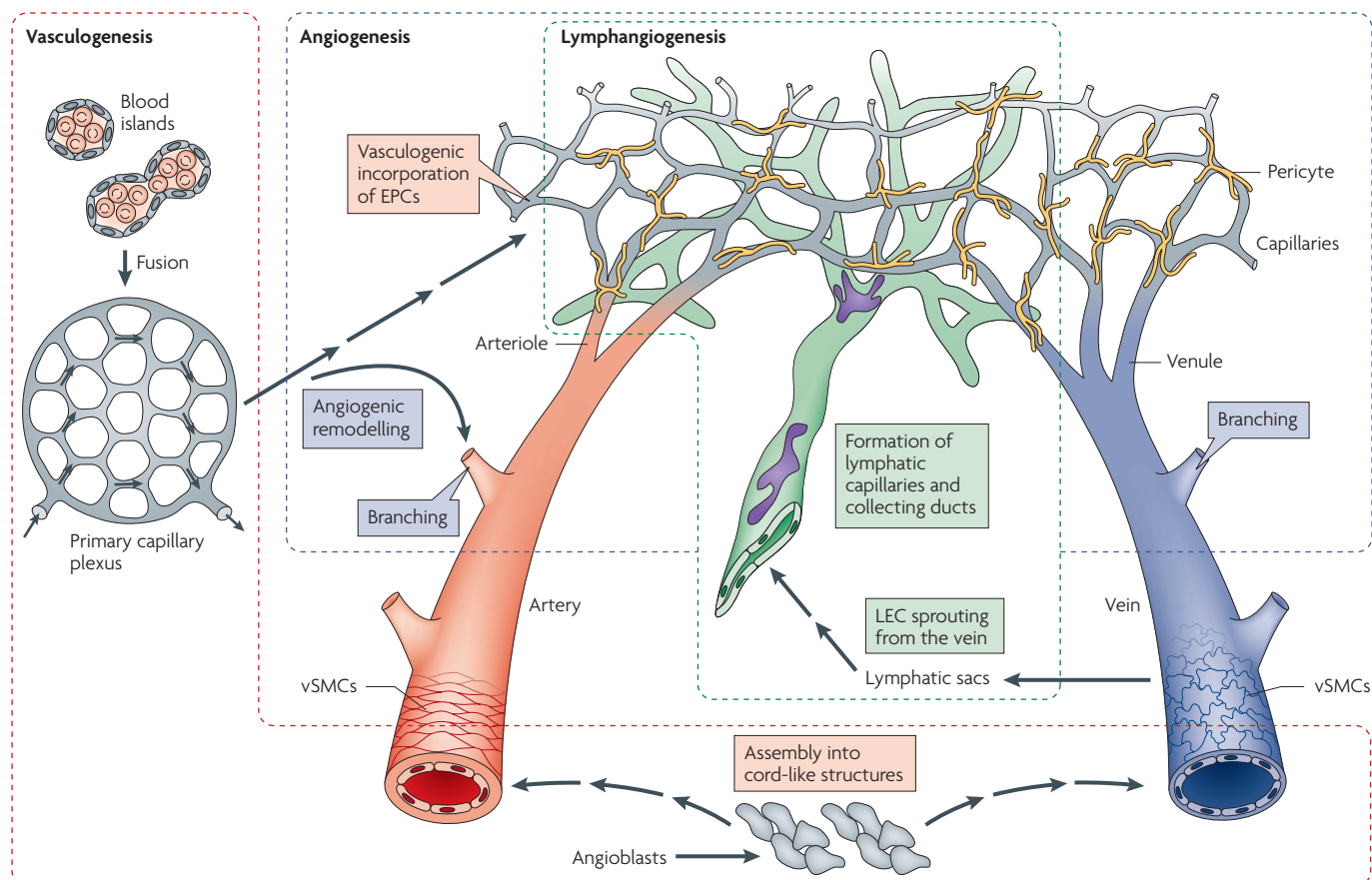doi:10.1038/nrm2183

Angiogenic growth of capillaries

Blood circulation proceeds from the heart through arteries into smaller arterioles and, finally, into capillary beds (FIG. 1). Capillaries form extensive networks that facilitate the exchange of gases and metabolic products before blood is returned through venules and veins to the heart and then on to the lungs to be replenished with oxygen. To increase blood transport into growing tissues, arteries and veins expand through circumferential growth and remodelling processes, whereas capillaries sprout and branch into larger, more complex networks. This transformation is most obvious early in embryogenesis when the first primitive and uniform vascular structures, known as primary capillary plexi, develop into hierarchically organized arteries, capillaries and veins (FIG. 1). Similar remodelling processes are thought to be important for the growth of blood vessels later in development as well as for physiological and pathological angiogenesis in the adult.

Stimulation of angiogenic growth. The most important molecule that controls blood-vessel morphogenesis is vascular endothelial growth factor A (VEGFA; also known as vascular permeability factor (VPF)), which is part of a large family of potent angiogenic regulators including placental growth factor (PlGF), VEGFB, VEGFC and VEGFD (also known as FIGF)[7,8]. VEGFA is required for the chemotaxis and differentiation of endothelial precursor cells (EPCs; angioblasts), EC proliferation, the direct assembly of ECs into vascular structures (vasculogenesis) and angiogenic remodelling (FIG. 1). Some of these roles are equally important for blood-vessel growth in adults and tumours.

The cellular responses to VEGF are tightly regulated by several mechanisms including expression of different family members, expression of alternatively spliced variants

Figure 1 | **Origin of endothelial cells and assembly of the vasculature.** Mesodermal cells in the early embryo differentiate into endothelial precursor cells (EPCs, angioblasts) and form aggregates, known as blood islands (left). Fusion of blood islands leads to the vasculogenic formation of honeycomb-shaped primary capillary plexi in the yolk sac and embryo itself. Blood circulation is established and primary plexi are remodelled into a hierarchical network of arterioles and arteries (red), capillaries (grey), and venules and veins (blue). The dorsal aorta and cardinal vein are directly formed through the assembly of angioblasts. The vasculogenic incorporation of circulating EPCs into growing blood vessels may contribute to regenerative or pathological neovascularization in the adult. Vascular smooth-muscle cells (vSMCs) are associated with arteries and veins, whereas capillaries are covered by pericytes (yellow). The first lymphatic endothelial cells (LECs) sprout from the embryonic veins, then migrate and form lymphatic sacs. Further steps of lymphangiogenic growth involve sprouting, branching, proliferation, differentiation and remodelling processes. The recruitment of lymphangioblasts from the adjacent mesenchyme has been speculated to be a further source of LECs. Blind-ending lymphatic capillaries (green) feed into collecting vessels and ducts. These larger lymphatics are sparsely covered by SMCs (purple) and contain valves that prevent backflow.

Mesoderm
The cell layer in the vertebrate embryo that differentiates into mesenchyme, connective tissue, bone, muscle, the cardiovascular system and blood cells.

Mesenchyme
Mesoderm-derived embryonic connective tissue that generates bone, cartilage, fibroblasts, smooth muscle and other cell types.

Pericytes
Mesenchyme-derived cells that cover blood vessels and make direct contact with endothelial cells through numerous long processes. Pericyte–endothelial interactions involve adhesion molecules and ion channels, and stabilize the endothelium.

and ligand binding to different receptors. For example, alternative splicing of *VEGFA* can generate several variants of the growth factor, including the so-called b isoforms that differ in the last six C-terminal amino-acid residues and have anti-angiogenic properties[9]. The balance between the different VEGFA isoforms can regulate blood-vessel growth and patterning. Binding of VEGFA to the receptor tyrosine kinase VEGFR2 (also known as KDR or FLK1) promotes EC differentiation, proliferation and sprouting. In embryos, this function is counteracted by another receptor, VEGFR1 (also known as FLT1), which has higher affinity for VEGF but weak tyrosine-kinase activity. VEGFR1 also exists in a secreted, catalytically inactive and therefore inhibitory isoform[7,8].

Many other factors influence the process of angiogenesis (FIG. 2). The local composition of the extracellular matrix (ECM) around the vasculature can affect angiogenesis positively or negatively[10]. The proteolytic release of pericellular matrix-bound VEGF isoforms is an important regulator of VEGF bioavailability and blood-vessel patterning[11]. Furthermore, the physical contact between ECs and pericytes is thought to induce a quiescent, non-sprouting phenotype[12,13].

Selection of ECs for sprouting. During angiogenic growth, some ECs within the capillary vessel wall are selected for sprouting (FIG. 2). These ECs are known as the tip cells, which lead the growing sprout. If all cells were to react indiscriminately, the affected section of the vascular network might disintegrate and thereby compromise tissue perfusion. Notch receptors and their Delta-like-4 (DLL4) ligand are essential for sprouting during mouse development. Reduced levels of DLL4 or blocking of Notch signalling enhances the formation of
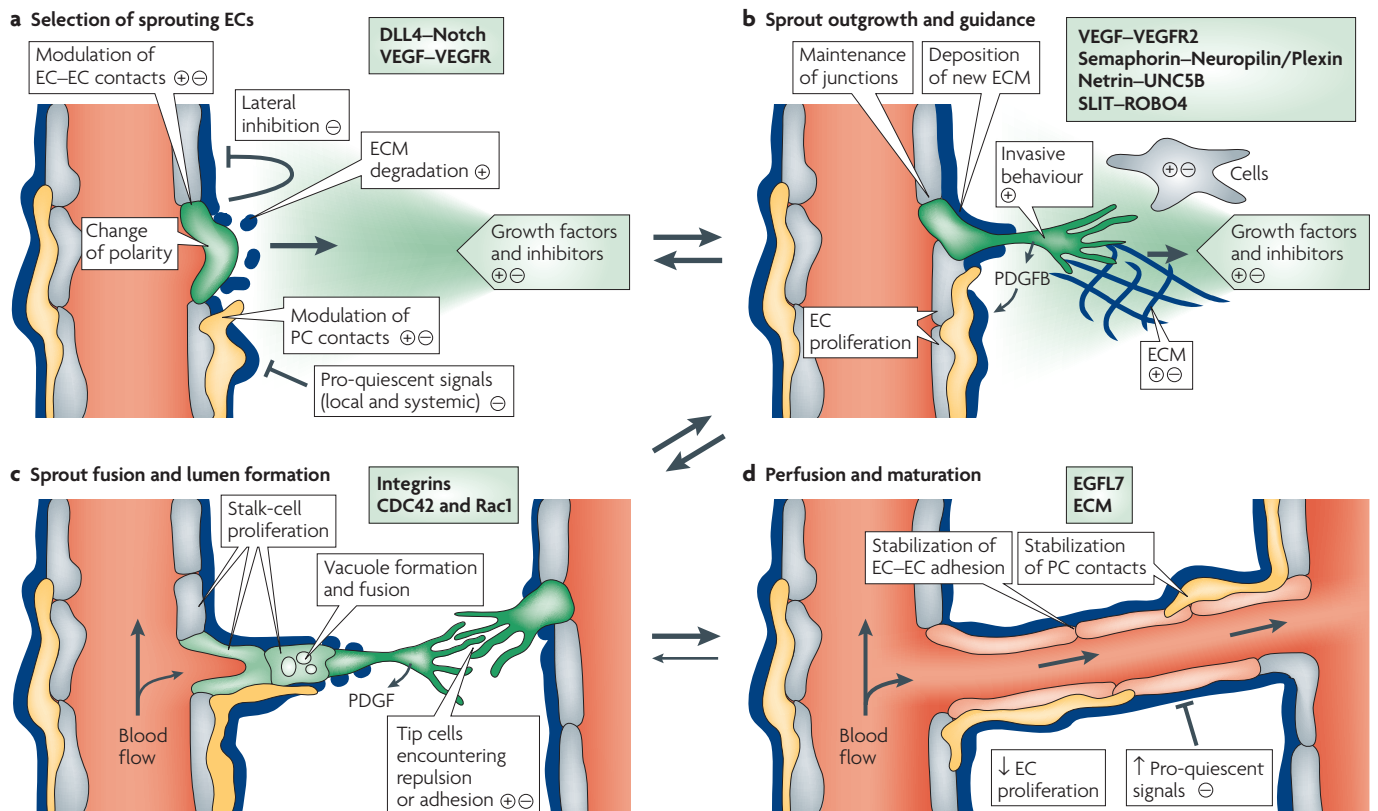
a | Selection of sprouting ECs

DLL4–Notch
VEGF–VEGFR

Modulation of EC–EC contacts ⊕⊖

Lateral inhibition ⊖

ECM degradation ⊕

Change of polarity

Growth factors and inhibitors ⊕⊖

Modulation of PC contacts ⊕⊖

Pro-quiescent signals (local and systemic) ⊖

b | Sprout outgrowth and guidance

VEGF–VEGFR2
Semaphorin–Neuropilin/Plexin
Netrin–UNC5B
SLIT–ROBO4

Maintenance of junctions

Deposition of new ECM

Invasive behaviour ⊕

Cells ⊕⊖

Growth factors and inhibitors ⊕⊖

PDGFB

EC proliferation

ECM ⊕⊖

c | Sprout fusion and lumen formation

Integrins
CDC42 and Rac1

Stalk-cell proliferation

Vacuole formation and fusion

PDGF

Blood flow

Tip cells encountering repulsion or adhesion ⊕⊖

d | Perfusion and maturation

EGFL7
ECM

Stabilization of EC–EC adhesion

Stabilization of PC contacts

Blood flow

↓ EC proliferation

↑ Pro-quiescent signals ⊖

Figure 2 | **Angiogenic sprouting. a** | Sprouting is controlled by the balance between pro-angiogenic signals (+), such as vascular endothelial growth factor (VEGF), and factors that promote quiescence (–), such as tight pericyte (PC; yellow) contact, certain extracellular matrix (ECM) molecules or VEGF inhibitors. In conditions that favour angiogenesis, some endothelial cells (ECs) can sprout (green), whereas others fail to respond (grey). Sprouting requires the flipping of apical–basal polarity, the induction of motile and invasive activity, the modulation of cell–cell contacts and local matrix degradation. **b** | The growing EC sprout is guided by VEGF gradients. Other signals may include attractive (+) or repulsive (–) matrix cues and guidepost cells in the tissue environment. Release of platelet-derived growth factor B (PDGFB) by the tip cells promotes the recruitment of PCs to new sprouts. EC–EC junctions need to be maintained after lumen formation to prevent excessive leakage. **c** | Adhesive or repulsive interactions that occur when tip cells encounter each other regulate the fusion of adjacent sprouts and vessels. Lumen formation in stalk ECs involves the fusion of vacuoles but other mechanisms may also contribute. **d** | Fusion processes at the EC–EC interfaces establish a continuous lumen. Blood flow improves oxygen delivery and thereby reduces pro-angiogenic signals that are hypoxia-induced. Perfusion is also likely to promote maturation processes such as the stabilization of cell junctions, matrix deposition and tight PC attachment. Growth factor withdrawal can trigger sprout retraction and endothelial apoptosis. DLL4, delta-like-4 ligand; EGFL7, epidermal growth factor ligand-7; ROBO4, roundabout homologue-4 (also known as magic roundabout); VEGFR2, VEGF receptor-2.

tip cells, resulting in dramatically increased sprouting, branching and fusion of endothelial tubes[14–19]. Similarly, endothelial sprouts fail to arrest their angiogenic motile behaviour and branch excessively when Dll4–Notch signalling is compromised in zebrafish embryos[20,21]. *Dll4* expression is induced in the tip cell, whereas the activation of Notch signalling in neighbouring ECs is thought to suppress sprouting of these cells[15,19,21] (FIG. 2).

The role of Notch is not confined only to physiological angiogenesis: disruption of DLL4–Notch interactions in tumour models causes extensive sprouting and branching of tumour blood vessels. However, such vessels are poorly functional, which leads to increased hypoxia, poor perfusion and decreased tumour growth. These findings suggest that the Notch pathway can provide a target for anti-angiogenic cancer therapy[16,17].

The mechanism by which Notch imposes differential behaviour on ECs that are exposed to similar pro-angiogenic stimuli appears to be directly connected to VEGFA signalling. *Dll4* expression is induced in response to VEGFA, whereas the activation of Notch by DLL4 suppresses the expression of *Vegfr2* and, in zebrafish, *vegfr3* (*flt4*)[14,17–19,21]. Therefore, *Dll4* expression might correspond to elevated VEGF-mediated signal transduction in selected ECs, whereas Notch activation in their direct neighbouring cells stifles pro-angiogenic responses (FIG. 2). Future work will need to address whether the Notch pathway is predominantly required in cells that are adjacent to the sprout tip or, perhaps in a dynamically regulated and transient fashion, also in the tip itself and other areas of the capillary bed.

Sprout outgrowth and chemotaxis. Despite insights into the mechanisms that select certain endothelial cells for outgrowth, very little is known about most aspects of the sprouting process. It seems obvious that sprouting ECs need to change their phenotype fundamentally, acquire invasive and motile behaviour, activate secreted or cell-surface-anchored proteases and locally degrade the subendothelial basement membrane. Interactions with surrounding ECs are likely to be modulated, presumably without complete disruption of cell–cell junctions and vessel-wall integrity (FIG. 2). The apical surface of ECs faces the vessel lumen, therefore apical–basal polarity needs to be reversed when new sprouts emerge from the outer (basal) side of the endothelium. Similarly, sprout elongation needs to be polarized and directional.

Consistent with the prominent expression of *VEGFR2* in the tips of sprouts, EC guidance is controlled by VEGFA. A spatial concentration gradient of a matrix-anchored isoform of VEGFA (VEGF164 in mice, VEGF165 in humans), which binds to heparan sulphate, functions as a chemoattractive signal that promotes the polarized extension of tip-cell filopodia. A shorter and freely diffusible VEGFA isoform (VEGF120 in mice, VEGF121 in humans) that lacks the heparan sulphate binding motif can support EC proliferation but not tip-cell guidance or proper vascular branching[22,23]. Neuropilins (NRP1 and NRP2) — transmembrane receptors that can bind to semaphorin family axon-guidance molecules and VEGF — might be involved in this differential response because NRP1 can bind to and enhance signalling through VEGF164 (VEGF165) but not VEGF120 (VEGF121)[8,24–26]. However, defects in vascular patterning and filopodia extension in *Nrp1*-knockout mice are relatively mild, which indicates that there are important roles for other VEGFRs in the tip-cell guidance process[22,27].

Growing vascular sprouts generate another concentration gradient, namely for platelet-derived growth factor B (PDGFB). In several developing tissues, high levels of PDGFB in tip cells promote the recruitment of pericytes that express the PDGF receptor β (PDGFRβ). This process ensures that the endothelium of growing vessels is sufficiently stabilized by supporting mural cells[23] (FIG. 3).

Common neurovascular guidance cues

Endothelial tip cells function as motile guidance structures that dynamically extend filopodia to explore attractive or repulsive signals that are present in the tissue environment. The dynamic nature, function and morphology of EC tips bear remarkable similarity to axonal growth cones and it has been proposed that nerves and blood vessels have evolved similar processes for the formation of complex networks[28,29].

Growing nerve fibres are guided to their targets by a combination of attractive or repulsive cues, such as certain matrix substrates, guidepost cells that provide information in a cell–cell contact-dependent fashion and soluble factors (FIG. 2). Some of these chemotactic signals can also attract endothelial sprouts. For example, the class 3 semaphorins control both axon guidance and vascular patterning (FIG. 3). Many semaphorins mediate

their effects by interacting with receptor complexes that are formed by (semaphorin-binding) neuropilins and (signal-transducing) plexin-family transmembrane proteins[30,31] (FIG. 3). At least one class 3 semaphorin, SEMA3E, can signal through plexin D1 independently of neuropilins[32]. Plexin D1 is expressed in ECs and its role in the pathfinding of blood vessels has been demonstrated by genetic experiments in mice and zebrafish[33,34]. Loss of plexin D1 causes aberrant sprouting of intersegmental vessels into SEMA3E-expressing somitic tissue, which suggests that semaphorins are repulsive cues for ECs[33].

Netrins are secreted matrix-binding proteins with homology to the basement-membrane molecule laminin (FIG. 3). In the nervous system, netrins are important axon-guidance cues, which can be either attractive, through interactions with receptors of the deleted in colorectal cancer (DCC) family, or repulsive, through receptors of the uncoordinated-5 (UNC5) family or UNC5–DCC heterodimers[28,29]. One of these receptors, UNC5B, is strongly expressed in capillaries and endothelial tip cells. Loss of *Unc5b* function in mice or fish leads to increased endothelial sprouting, whereas stimulation of ECs with the ligand netrin-1 leads to the retraction of tip-cell filopodia[35], which is consistent with a negative regulation of blood-vessel growth by netrins and UNC5B. However, some evidence suggests that netrins could promote developmental and therapeutic angiogenesis, which may involve as-yet-unknown receptors[36].

SLIT proteins form another family of secreted axon-guidance molecules that might have a role in EC sprouting. Interactions of SLIT proteins with roundabout (ROBO) receptors (FIG. 3) repel axon growth cones and inhibit the migration of certain cell types. ROBO4 (magic roundabout) is essential for normal vascular patterning, although it is controversial whether ROBO4 is capable of direct binding to any of the three known mammalian SLIT proteins[37–39].

These examples highlight that endothelial sprouts use similar attractive and repulsive tissue-derived navigational cues as growing nerve fibres to control the patterning and directional growth of the vascular network.

Transformation of sprouts into vessels

Many further steps are required to convert endothelial sprouts into functional, blood-carrying vessels (FIG. 2). For example, sprout extension can involve the migration of the endothelial cells behind the tip. Such chains of migrating ECs are visible during the growth of intersegmental vessels in the trunk of zebrafish embryos[20,21,40]. Alternatively, local EC proliferation can promote sprout extension. For example, in the developing mouse retina, ECs in the stalk of sprouts proliferate whereas the tip cells do not[23]. However, only the leading tip cell undergoes a single cell division in zebrafish intersegmental vessels[21]. These differences in the mitotic pattern together with higher levels of *Dll4*, *Vegfr2* and *Pdgfb* transcripts and the growth-cone-like morphology of sprout tips indicate that ECs at the tip and in the stalk of sprouts are different. However, a fraction of the stalk cells

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Filopodia
Slender cellular processes that extend from the front of migrating cells, attach to the surrounding matrix and help to move cells forward.

Mural cells
Cells of the outer vessel wall: pericytes and vascular smooth-muscle cells.

Axonal growth cones
Dynamic guidance structures at the distal end of growing nerve fibres that direct fibres to their appropriate targets and thereby promote the correct 'wiring' of the nervous system.
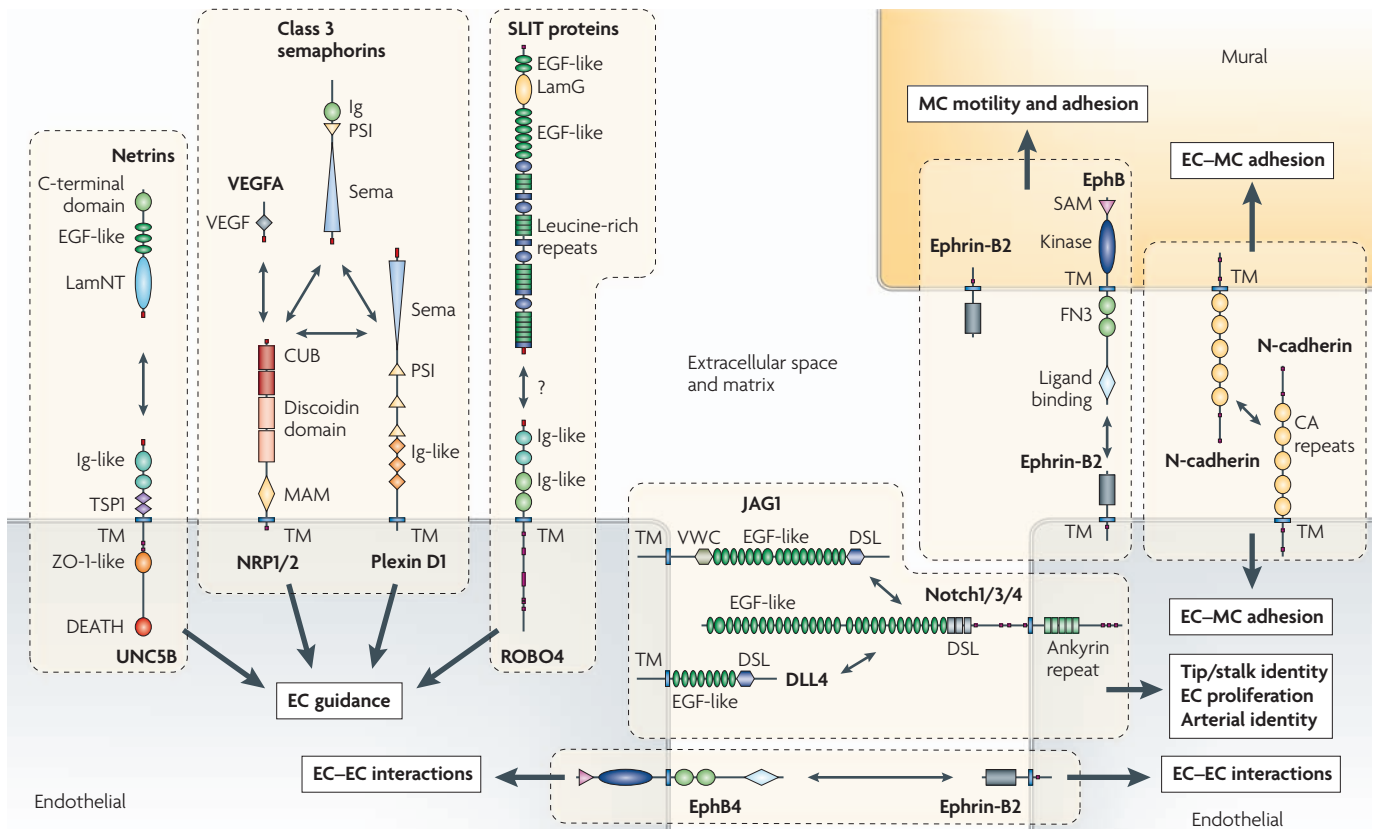
Figure 3 | **Guidance cues, adhesion molecules and cell-fate regulators that function both in the nervous system and in the vasculature.** Black arrows indicate molecular interactions. Navigational cues that direct growing axons such as netrins, semaphorins and SLITs also guide endothelial cells through their corresponding receptors. The specificity of SLIT-ligand binding to ROBO4 is controversial[38,39]. Notch signalling triggered by DLL4 or JAG1 controls endothelial sprouting, proliferation and arteriovenous identity. Binding of ephrin-B2 — a guidance cue and a modulator of neuronal activity — to its receptor EphB4 modulates endothelial cell (EC)–EC interactions and is essential for angiogenesis. Ephrin-B2 expression in mural cells (MC) controls their motility and adhesion[105]. This function may be modulated by endothelial ephrin-B2. The homophilic cell-adhesion molecule N-cadherin promotes synapse formation between neurons and stabilizes the EC–pericyte contact[119,146,147]. CA, cadherin repeats; CUB, cubilin-related; DLL4, Delta-like-4 ligand; DSL, Delta/Serrate/Lag-2; EGF-like, epidermal-growth factor like; FN3, fibronectin type 3-like; Ig, immunoglobulin; JAG1, Jagged1; LamG, laminin globular G domain; LamNT, laminin N-terminal domain; MAM, meprin–A5-protein–PTPmu-related; PSI, plexin–semaphorin–integrin; ROBO4, roundabout homologue-4; SAM, sterile-α motif; Sema, semaphorin; TM, transmembrane; TSP1, thrombospondin-1 like; UNC5B, uncoordinated-5 homologue B; VWC, von Willebrand type C factor-like; ZO-1-like, zona occludens-1-like.

also extend filopodia and show elevated expression of *Dll4* or *Pdgfb*. It remains unclear whether the differences between tip and stalk ECs reflect stable, genetically controlled fates or transient adaptations to positional cues within the sprout.

To form new vascular connections, tip cells need to suppress their motile, explorative behaviour upon encountering their targets, the tips of other sprouts or existing capillaries. Strong adhesive interactions and EC–EC junctional contacts need to be established at this joining point. The failure to do so (for example, because of repulsive signalling triggered by cell–cell contact) would presumably preclude the generation of a blood-transporting vessel by these ECs (FIG. 2). Such a process might also help to prevent the fusion between incompatible parts of the vasculature and the formation of arteriovenous shunts.

The establishment of blood flow requires the formation of a vascular lumen, which may occur within growing endothelial sprouts before or after they have joined with other vessels (FIG. 2). Cellular structures can be converted into tubes in various ways, as has been shown for epithelial tissues in vertebrates and invertebrates[41]. Although little is known about the lumenization of blood vessels, there is strong evidence for the involvement of pinocytosis ('cell drinking') and vacuole formation. Studies in cultured ECs suggest that these processes require the integrin cell-matrix adhesion machinery and the small GTPases CDC42 and Rac1 (REF. 42). High-resolution time-lapse imaging of growing intersegmental vessels in zebrafish has established that the lumen in these ECs is formed by intracellular and, subsequently, intercellular fusion of large vacuoles[40].

Pinocytosis
Uptake of extracellular liquid into cells in the form of membrane-coated vesicles.

a Circulating cells and blood-vessel growth
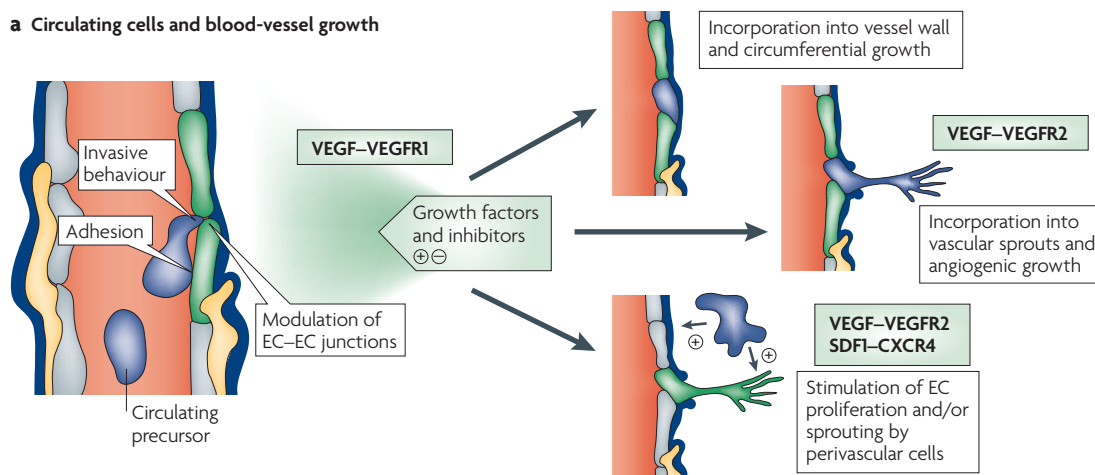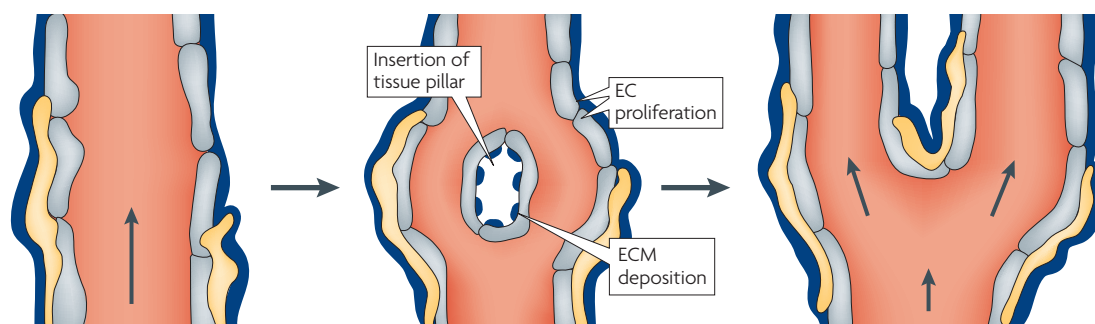

b Vessel splitting by intussusception

Figure 4 | **Vasculogenic and intussusceptive growth of blood vessels. a** | Recruitment and incorporation of cells from the blood circulation requires positive chemotactic signals and local factors that promote adhesion to endothelial cells (ECs). Transendothelial migration and/or the incorporation of precursor cells into the endothelium require the modulation of existing EC–EC contacts. Circulating precursor cells (angioblasts) have been suggested to differentiate into ECs and lead to an expansion of the vessel diameter (circumferential growth) or, alternatively, they may be recruited to new angiogenic sprouts. Circulation-derived cells do not necessarily acquire an endothelial fate but can instead promote the proliferation and sprouting of local ECs from a perivascular location. **b** | Intussusception — the splitting of vessels through the insertion of tissue pillars — is a separate mechanism that leads to the expansion of blood vessels. Little is known about the function or regulation of intussusceptive growth but the process is likely to involve EC proliferation, cell movement, the degradation of extracellular matrix (ECM) and the deposition of new ECM molecules. CXCR4, CXC-chemokine receptor-4; SDF1 (also known as CXCL12), stromal cell-derived factor-1; VEGF, vascular endothelial growth factor; VEGFR1, VEGF receptor-1.

Lumen formation and tubulogenesis are also controlled by EGF-like domain-7 (EGFL7; also known as vascular endothelial statin), a small secreted ECM-associated protein that is expressed by the ECs. Although the exact role of EGFL7 requires further investigation, it appears to suppress matrix adhesion without affecting EC proliferation[43].

The generation of a lumen and the onset of blood flow may help to stabilize new vascular connections. Improved oxygen delivery lowers local VEGFA expression. The recruitment of pericytes, the deposition of ECM proteins into the subendothelial basement membrane and reduced EC proliferation also promote vessel maturation and quiescence (FIG. 2). At this stage, as well as during other steps in the sprouting process, changes in the local balance between pro- and anti-angiogenic factors may lead to the elimination (pruning) of the new connections; for example, by EC retraction or apoptosis. The pruning process is most extensive around the oxygen-rich arteries in the developing retina, consistent

with the suppression of hypoxia-driven VEGFA expression. Treatment of experimental tumours with VEGFA inhibitors has shown that empty sleeves of basement membrane with associated pericytes remain when immature vessels are eliminated. Because these structures favour the rapid regrowth of blood vessels when the VEGF signal is restored, matrix sleeves might be potential targets in cancer therapy[44].

Non-angiogenic growth of blood vessels

Angiogenic sprouting of ECs from existing blood vessels is not the only mechanism that contributes to vascular growth. For example, the dorsal aorta and cardinal vein in the early embryo are formed by vasculogenesis — the direct assembly of angioblasts and ECs[45]. Similarly, bone-marrow-derived circulating EPCs are thought to contribute to vasculogenic regenerative or pathological blood-vessel growth in the adult[46]. Such incorporation of EPCs into the endothelial monolayer could lead to circumferential enlargement of vessels.

Alternatively, recruitment of EPCs to growing endothelial sprouts may combine vasculogenic and angiogenic mechanisms (FIG. 4). In most cases, however, the contribution of EPCs to angiogenesis is small.

VEGF and its receptors are crucial for the mobilization of EPCs in the bone marrow and their recruitment to specific sites in the body. In both processes, *VEGFR1* expression and signalling in bone-marrow-derived cells have essential roles even though the same receptor inhibits angiogenesis during embryogenesis[7,8,46]. VEGF–VEGFR1 signalling also mediates the recruitment of bone-marrow-derived monocytic cells, which do not give rise to ECs and are not incorporated into the endothelium[7,47]. For example, recruited bone-marrow-derived circulating cells (RBCCs), which express CXC-chemokine receptor-4 (CXCR4), are retained in the perivascular space. This retention is due to the local expression of its ligand stromal-derived factor-1 (SDF1) in response to VEGFA. The recruitment of RBCCs enhances endothelial proliferation and angiogenesis, whereas interfering with RBCCs attenuates VEGF-mediated pro-angiogenic effects[47] (FIG. 4a).

Finally, it should be mentioned that the vascular network can be locally extended by intussusception — the splitting of blood vessels through the insertion of tissue pillars[48] (FIG. 4b). Despite strong morphological evidence that supports a role for this process in various tissues, little is known about the physiological role and molecular regulation of intussusception.

Arteriovenous differentiation

During the remodelling of the primary capillary plexi in the early embryo, arteries and veins are formed in a process that is known as arteriovenous differentiation[3,49]. The growth and specialization of arteries and veins continues throughout development and reflects the distinct haemodynamic properties within the vascular network. Concentric layers of contractile vascular smooth-muscle cells (vSMCs), ECM and elastic fibres allow arteries to resist shear stress and pulsatile flow (FIG. 5). Elevated fluid shear stress is also a main factor that mediates the enlargement and remodelling of existing arteries and arterioles (arteriogenesis) in the adult[3,50]. The venous low-pressure system contains valves, which are flap-like structures that open unidirectionally to prevent backflow (FIG. 5). Veins are enveloped by fewer vSMCs but even this low degree of coverage is physiologically relevant, as shown by the degeneration of the vSMC–matrix layer in varicose veins and some other venous diseases[51,52].

Notch controls arterial differentiation. From the earliest stages of cardiovascular development, arterial ECs share the expression of certain transcription factors, signalling molecules, gap-junction proteins, ECM molecules and adhesion proteins that are largely absent from veins. Some of these molecules reflect the specific physiological needs of arteries, whereas others, such as those in the Notch pathway, are directly involved in the differentiation of the arterial branch[49]. Proteolytic processing of activated Notch releases an intracellular fragment, which translocates to the nucleus and associates with

transcriptional regulators of the suppressor of hairless family (CBF1 or RBP-Jκ in vertebrates). This complex allows the expression of basic helix–loop–helix (bHLH) proteins of the hairy/enhancer-of-split-related transcription factor family — Hey2 and Her in zebrafish, and Hey and Hes in mammals — which, in turn, control the expression of other downstream target genes[53,54].

Knockout mice that lack components of the Notch pathway — NOTCH1 and/or NOTCH4, the ligands Jagged1 (JAG1) or DLL4, presenilin proteins that are required for Notch proteolytic processing, the ubiquitin-ligase mind bomb (a regulator of ligand endocytosis and Notch signalling), the transcription factor RBP-Jκ or the Hey family members HEY1 and HEY2 — have severe vascular defects and are embryonic lethal[55–62]. Although it is likely that some or all of these molecules are also required for endothelial sprouting and capillary growth, the knockout phenotypes and their defective arteriovenous-marker expression support important roles for these molecules in arterial differentiation. Similarly, manipulation of Notch signalling or the *hey2* gene in zebrafish embryos shows that Notch signalling is necessary and sufficient for the expression of arterial markers[49].

Arterial markers and arteriovenous identity. Probably the best-known arterial EC marker is the transmembrane protein ephrin-B2 (encoded by *EFNB2*) which is a highly promiscuous ligand that can bind to at least six different receptors within the large Eph tyrosine kinase family[63,64]. Expression of the cognate receptor (EphB4) is largely confined to the venous endothelium so that ephrin-B2 and EphB4 are frequently used as molecular markers for the identification of arteries and veins. Although ephrin-B2 and EphB4 are essential for angiogenesis in the early mouse embryo, their precise function in the vasculature and their potential involvement in the arteriovenous-fate decision are unclear[28,29].

VEGF is also an important regulator of arterial differentiation. Exposure of cultured ECs to VEGFA leads to increased *Dll4* expression, Notch signalling and *ephrin-B2* upregulation[65,66] (FIG. 5). In mouse embryonic skin, VEGFA secreted by peripheral nerves controls arterial differentiation[67]. Similarly, the expression of Notch pathway genes and *ephrin-B2* is regulated by VEGFA in zebrafish[49]. How can VEGFA simultaneously control EC proliferation, vasculogenesis, angiogenesis and arterial differentiation? Local differences in VEGFR expression and signalling are possible explanations. In the zebrafish, Vegfr2 is abundant in the dorsal aorta, whereas Vegfr3 (Flt4) is predominantly found in veins[49].

Neuropilins also show distinct arteriovenous-specific patterns. NRP1 is found in arteries, whereas NRP2 is restricted to veins and lymphatic vessels[68–70]. The two neuropilins can bind to different VEGF isoforms, which may lead to distinct signalling responses in arteries and veins[8,29]. Local regulation might also involve *Vegf* splicing, the availability of heparan sulphate proteoglycans, proteolytic processing of VEGF and the modulation of its matrix-binding properties[11,71].

Vascular smooth-muscle cells
Specialized smooth-muscle cells that form the outer layer of arteries, arterioles and larger veins. They provide blood vessels with mechanical stability that is due to their contractile properties and the deposition of matrix and elastic fibres.
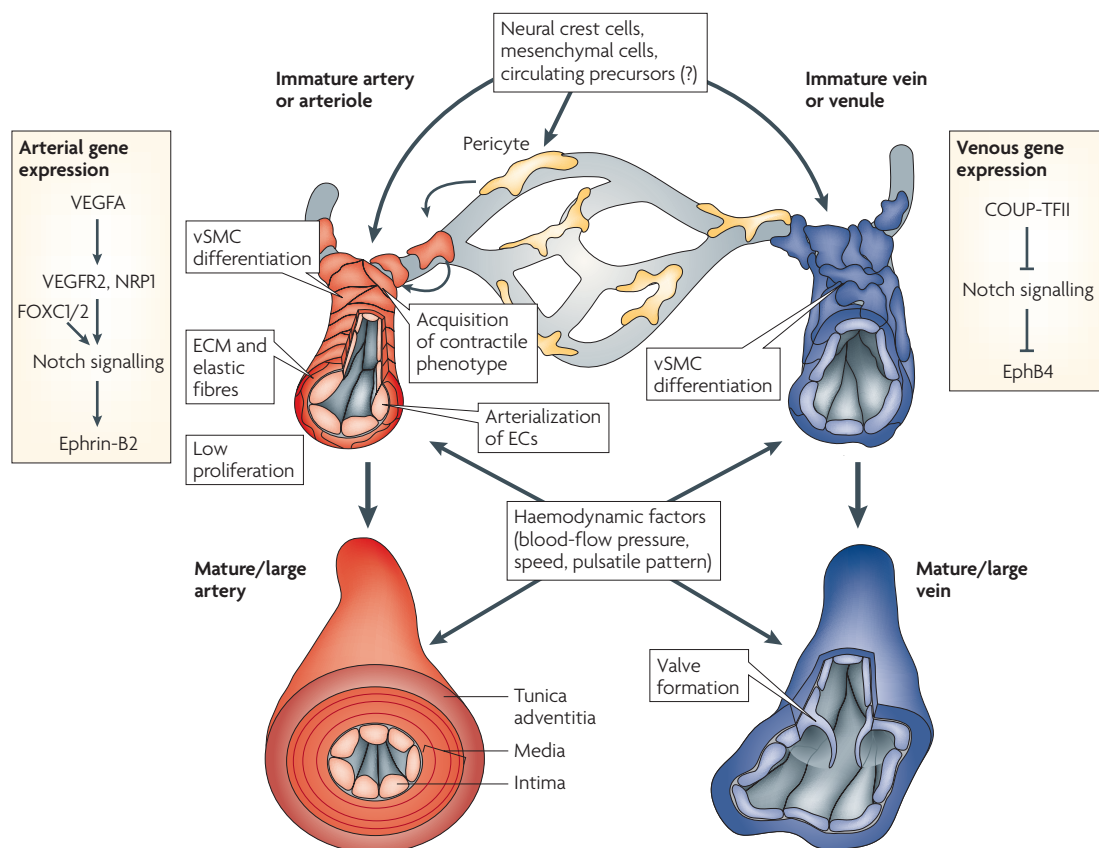
Neural crest cells, mesenchymal cells, circulating precursors (?)

Immature artery or arteriole

Immature vein or venule

Pericyte

Arterial gene expression

VEGFA

VEGFR2, NRP1

FOXC1/2

Notch signalling

Ephrin-B2

Venous gene expression

COUP-TFII

Notch signalling

EphB4

vSMC differentiation

ECM and elastic fibres

Acquisition of contractile phenotype

Arterialization of ECs

vSMC differentiation

Low proliferation

Mature/large artery

Haemodynamic factors (blood-flow pressure, speed, pulsatile pattern)

Mature/large vein

Tunica adventitia

Media

Intima

Valve formation

Figure 5 | **Arteriovenous differentiation and mural-cell recruitment.** Arterial endothelial cells (ECs) have a characteristic spindle-like morphology and alignment in the direction of blood flow, low proliferation, and a characteristic gene-expression profile. Various genes, such as the arterial marker ephrin-B2, are induced by vascular endothelial growth factor A (VEGFA, which can signal through VEGF receptor-2 (VEGFR2) or neuropilin-1 (NRP1)) and stimulate Notch signalling[49]. The forkhead box transcription factors FOXC1 and FOXC2 promote this process by increasing the expression of the Notch ligand Delta-like-4 (DLL4)[75]. Expression of components of the Notch pathway in the venous endothelium is suppressed by the nuclear orphan receptor COUP-TFII (REF. 74). This block permits the production of the venous marker EphB4. Arteries are surrounded by extensive extracellular matrix (ECM), elastic fibres and layers of contractile vascular smooth-muscle cells (vSMCs). The wall of mature large arteries (such as the aorta) is composed of the inner tunica intima (ECs and basement membrane), the central tunica media with multiple alternating layers of matrix and vSMCs, and the outermost layer tunica adventitia, which is rich in collagen and fibroblasts. Veins have less extensive vSMC coverage and contain valves. Haemodynamic factors control arteriovenous differentiation and morphological changes in ECs and vSMCs[72,73]. The mural cells that cover arteries, veins and capillaries are derived from mesenchymal or neural crest cell precursors and, possibly, from circulating cells. vSMCs undergo several steps of differentiation before they acquire their mature morphology and gene-expression profile.

Blood-flow dynamics also regulate arteriovenous differentiation. Arterial-type pulsatile, high-pressure flow will lead to the morphological and molecular arterialization of blood vessels in the adult[72]. Conversely, changing arterial to venous flow in the yolk sac of chick embryos suppresses the normal arterial expression of *NRP1* and *ephrin-B2* (REF. 73). These data indicate that the haemodynamic and genetic regulation of blood-vessel morphogenesis are closely integrated (FIG. 5).

Venous differentiation. COUP-TFII, a member of the orphan nuclear receptor superfamily, is expressed in the venous endothelium and functions upstream in the arteriovenous decision-making process (FIG. 5). In mice that lack COUP-TFII (encoded by the gene *Nr2f2*), veins acquire arterial characteristics and express components of the Notch pathway and ephrin-B2. Conversely, mis-expression of COUP-TFII in arterial ECs leads to the downregulation of arterial markers and the expression of venous genes[74]. Presumably independently of COUP-TFII, the two forkhead box transcription factors FOXC1 and FOXC2 are also required for arterial identity, normal vascular morphogenesis and expression of Notch-pathway components[75] (FIG. 5).

Local specialization of ECs. The differences between arteries, capillaries, veins and lymphatics show that ECs form numerous subpopulations. In fact, organ-specific specialization of the vasculature requires an even larger degree of EC heterogeneity in terms of gene expression. For example, the endothelium of the central nervous system forms a barrier (termed the blood–brain barrier)

Podocyte
Specialized, highly branched epithelial cell in the filtering units (glomeruli) of the kidney. Numerous podocyte foot processes cover the glomerular capillary basement membrane and thereby form a size-selective filtration barrier that is permeable to water, salts and glucose but retains macromolecules in the bloodstream.

Lymphoedema
Harmful interstitial liquid accumulation that is due to insufficient lymphatic drainage.

that tightly controls transendothelial transport and cell migration. By contrast, vessels in endocrine glands and the pancreas, intestine and kidney are highly permeable owing to pore-like fenestrations, which can be induced by VEGF or endocrine gland–VEGF (EG–VEGF), a tissue-specific angiogenic regulator[76]. Also, the formation of valves in lymphatic vessels and veins most probably involves as-yet-unidentified instructive signals recognized by a few ECs but not their neighbouring cells. The identification and characterization of such local signals are important areas for future research.

Growth and identity of lymphatic vessels

The lymphatic vasculature forms a unidirectional network of blind-ended capillaries (terminal lymphatics) that collect the protein-rich fluid that exudes from blood vessels and then drain through a conduit system of collecting vessels, lymph nodes, lymphatic trunks and ducts into the venous circulation[2,4,77,78]. Loss of lymphatic function in humans as a result of hereditary disease, lymphatic-vessel damage or surgical removal of lymph nodes causes lymphoedema[4]. The lymphatic network is also involved in the immune surveillance of the body. Lymphocytes and antigen-presenting dendritic cells travel from peripheral tissues through lymphatic vessels to the lymphoid organs, in which immune defences against pathogens are mounted.

Although the ECs of blood vessels (BECs) and lymphatics (LECs) share many features, such as their flat shape, strong apical–basal polarity and the expression of certain endothelial markers, the distinct functional roles of the two vascular networks require extensive specialization. The endothelium of terminal lymphatics lacks a continuous basement membrane and LEC–LEC contacts are not tightly sealed by junctional complexes. Owing to anchoring filaments that link LECs to the interstitial ECM, fluid accumulation in the tissue opens intercellular gaps and enhances the uptake of interstitial fluid. The distinct architecture and function of lymphatic vessels requires some specific gene products that are not shared with the BECs[2,79] (BOX 1). Because the mammalian lymphatic system originates from the embryonic veins, its morphogenesis involves the acquisition of LEC identity and separation from the blood-vessel network (FIG. 6). A small part of the LEC population may be generated from mesenchymal or circulating precursor cells[80,81], but little is yet known about these processes (FIG. 6).

LEC identity and separation from blood vessels. In mouse mid-gestation embryos, a distinct cluster of ECs on the dorsal side of the jugular vein express the prospero-related homeobox-1 (PROX1) transcription factor and differentiate into the first LECs (FIG. 6). The activity that induces the local expression of this transcription factor is unknown but the role of PROX1 in the upregulation of many lymphatic-specific genes and suppression of certain blood-vessel markers has been firmly established by gene-expression profiling studies in cultured cells[79,82].

PROX1 function is also essential for lymphatic development *in vivo* (FIG. 6). Inactivation of *Prox1* in mice results in defective extension of ECs from the cardinal vein, loss of lymphatic-marker expression and lack of the lymphatic vasculature[83,84]. PROX1 function is not confined to the onset of lymphatic development. Heterozygous *Prox1*-mutant mice are viable but have mispatterned, dysfunctional and ruptured lymphatic vessels. This defect is most obvious in the gastrointestinal system and leads to obesity because leaked lymph promotes the differentiation of adipocytes[85]. The expression of *Prox1* in the LECs of the small intestine is severely reduced in mice that lack fasting-induced adipose factor (FIAF; also known as ANGPTL4). *Fiaf* mutants die within a few weeks of birth with dilated and blood-filled lymphatics that are aberrantly connected to blood vessels[86].

The critical importance of the separation of blood vessels and lymphatic vessels is also demonstrated by studies in mice that lack the cytoplasmic tyrosine kinase SYK or the adaptor protein SLP76 (REFS 81,87). Both mutants develop abnormal connections between BECs and LECs during embryogenesis.
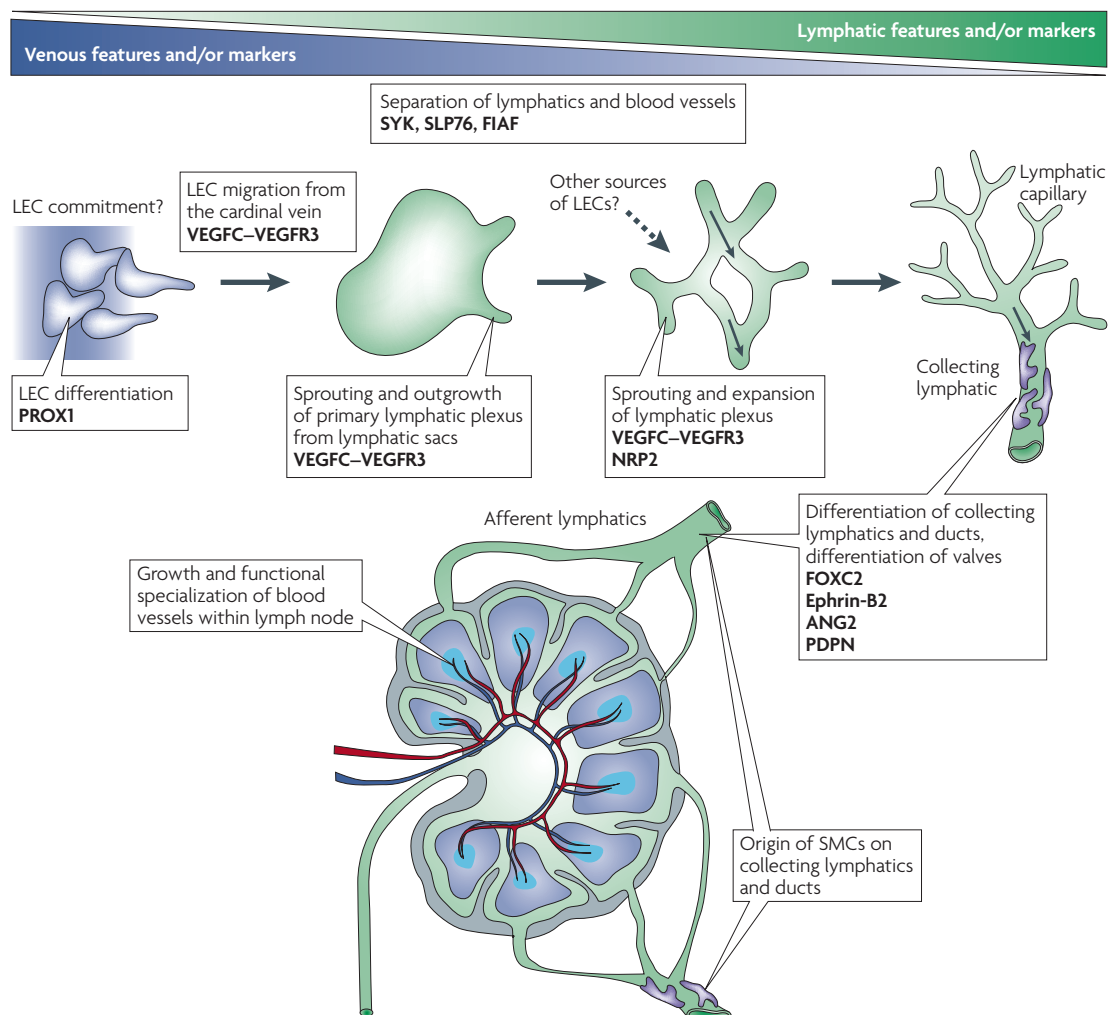
Figure 6 | **Developmental lymphangiogenesis.** Lymphatic endothelial cells (LECs) acquire their specialized gene-expression profile progressively during the formation of the lymphatic network. Expression of the prospero-related homeobox-1 (PROX1) transcription factor marks the first LECs within embryonic veins. The tyrosine kinase SYK and the adaptor protein SLP76 are necessary for the separation of blood and lymphatic vessels, whereas fasting-induced adipose factor (FIAF) is required for *PROX1* expression in the intestine. Assembly of lymphatic sacs and sprouting processes lead to the formation of lymphatic plexi, which will expand and remodel into a highly branched network of distal capillaries, collecting lymphatic vessels and ducts. Vascular endothelial growth factor receptor-3 (VEGFR3) signalling in LECs is important throughout lymphangiogenic growth and may be enhanced by neuropilin-2 (NRP2). The forkhead box transcription factor FOXC2, the mucin-type sialoglycoprotein podoplanin (PDPN) and the ligands ephrin-B2 and angiopoietin-2 (ANG2) are involved in the regulation of these late differentiation steps. LECs have also been suggested to differentiate from other sources such as mesenchymal cells or circulating precursors. SMCs, smooth-muscle cells.

Surprisingly, these defects reflect a requirement for SYK and SLP76 activity in circulating cells, presumably haematopoietic-system-derived EPCs[81,87]. The mechanistic role of SYK and SLP76 in this process remains unclear but both molecules are involved in integrin signalling and could help to direct circulating precursor cells to specific vascular beds — for example, by modulating their adhesion or migration.

In the context of blood-vessel–lymphatic separation, it is noteworthy that LECs and BECs show limited intermingling during co-culture[79]. This suggests that the two cell types have distinct cell–cell adhesion properties, which might help to prevent cell mixing and shunt formation between the two vascular networks.

LEC sprouting and proliferation. Mirroring the crucial role of VEGFA in the growth of blood vessels, VEGFC functions as an important regulator of lymphangiogenesis (FIG. 6). VEGFC promotes the sprouting of the first PROX1-positive LECs from the jugular vein and induces LEC proliferation and survival[88]. Accordingly, *Vegfc*-knockout mice fail to form primary lymph sacs, lack all lymphatic vessels, develop severe oedema and die before birth. Even the loss of a single *Vegfc* allele in heterozygous mutants leads to lymphatic-vessel hypoplasia and lymphoedema in the skin[88].

VEGFD also has lymphangiogenic activity but is not crucial for lymphatic development[89]. VEGFC and VEGFD signal mainly through VEGFR3 but certain proteolytically

processed forms can also interact with VEGFR2 (REFS 4,90). These binding properties and expression of *Vegfr3* in BECs explain the severe vasculogenic and angiogenic defects during early embryogenesis in VEGFR3 loss-of-function models[91,92]. Perinatally, *Vegfr3* expression becomes increasingly confined to the lymphatic vasculature so that disruption of VEGFC–VEGFR3 signalling selectively compromises lymphangiogenesis[93,94]. However, some inhibition of angiogenesis in tumours and wounds has also been observed, and correlates with the re-expression of *Vegfr3* in blood vessels[95].

NRP2, which is expressed in lymphatic vessels, can interact with VEGFR3 and bind to VEGFC and VEGFD, and is essential for lymphangiogenesis[68,96]. *Nrp2*-knockout mice show reduced LEC proliferation and fail to develop small-diameter lymphatic vessels and capillaries[68].

VEGFA also stimulates lymphatic growth in several experimental systems[97–99] but this activity might be indirect (for example, through the recruitment of inflammatory cells and increased *VEGFC* expression[100,101]). Other growth factors and chemokines that have been suggested to have lymphangiogenic activity include fibroblast growth factor (FGF), hepatocyte growth factor (HGF), PDGF, insulin-like growth factor-1 (IGF1) and IGF2 (REF. 2). However, some of these molecules may stimulate lymphatic growth indirectly or only in pathological settings.

Differentiation of lymphatic vessels

Little is known about the lymphatic maturation programme but known processes include the gradual suppression of LEC proliferation and sprouting, protection against VEGFC withdrawal, valve formation and the differentiation of the network into lymphatic capillaries and smooth-muscle-covered collecting lymphatics, lymphatic trunks and ducts (FIG. 6).

One important regulator of these processes is VEGFC, which remains essential until developmental lymphangiogenesis is completed postnatally[93]. The patterning of the lymphatic network and SMC recruitment to the collecting lymphatics are defective in angiopoietin-2 *(Ang2)*-deficient mice. ANG1 can also promote lymphangiogenesis, trigger LEC proliferation and rescue the lymphatic defects of *Ang2*-knockout mice[102,103].

Similar to the angiopoietins, ephrin-B2 is involved in the angiogenic growth of both blood vessels and lymphatic vessels, as shown by LEC sprouting, lymphatic patterning and valve-formation defects in mutant mice[104]. A further interesting feature of the *ephrin-B2* mutant is the appearance of ectopic SMC coverage on cutaneous lymphatic capillaries (FIG. 7). Although this is caused by a cell-autonomous differentiation defect of the lymphatic endothelium, SMCs are also found on terminal lymphatics in mice that lack *ephrin-B2* in mural cells. In these mutants, pericytes and vSMCs fail to associate stably with blood vessels and some migrate to lymphatics[105] (FIG. 7). Lymphatics also acquire ectopic vSMCs in *Pdgfrb*-knockout mice, which suggests that intact pericyte and vSMC chemotaxis helps to ensure that only the correct vessels acquire mural cells[105].

Abnormal association of smooth-muscle actin-positive cells with lymphatic vessels is associated with compromised tissue drainage in lymphoedema distichiasis (LD), a congenital disease caused by inactivating, autosomal dominant mutations in the human *FOXC2* gene[106–108]. Similarly, *Foxc2*-deficient mice have dysfunctional lymphatics that express several BEC markers including PDGFB, are covered by smooth-muscle cells, and lack valves (FIG. 7). Normal early lymphatic development in these mutants indicates that FOXC2 is mainly required for the late maturation steps[108].

Maturation of blood vessels

The term maturation describes the stepwise transition from an actively growing vascular bed to a quiescent, fully formed and functional network. This involves the suppression of endothelial proliferation and sprouting, protection against growth-factor (VEGF) withdrawal, stabilization of existing vascular tubes, cellular-differentiation processes such as the formation of valves, fenestrations or tight-junction barriers, and the incorporation of mural cells into the vessel wall (see below)[3,45]. Maturation is frequently compromised in pathological settings, particularly in the vasculature of tumours[109,110].

A prominent and widely recognized feature of the maturation programme is the recruitment of mural cells — pericytes and vSMCs (FIG. 5). Pericytes establish direct cell–cell contact with ECs and cover capillaries and immature blood vessels, whereas vSMCs cover mature and larger diameter vessels, such as arteries and veins, and are separated from the endothelium by a basement-membrane layer. Both mural cell types share a mesenchymal, fibroblast-like morphology and their phenotypes are possibly interconvertible, although this has not been formally demonstrated. Understanding the relationships between mural cells is complicated by the difficulty in identifying pericytes. Molecular markers such as the proteoglycan NG2, the intermediate filament protein desmin, α-smooth-muscle actin, the regulator of G-protein signalling RGS5 or the reporter transgene *X-LacZ4* label only subsets of the highly heterogeneous pericyte population and are also expressed by vSMCs and other cell types[12,13]. Furthermore, it remains unclear whether there are other, as-yet-unidentified and perhaps tissue-specific subsets of these cells.

PDGFRβ cooperates with G-protein coupled receptors. The ontogeny of pericytes and vSMCs is similarly puzzling and complex (FIG. 5). Some mural cells are derived from neural crest cells that delaminate from the neural tube early in embryogenesis, whereas others descend from undifferentiated mesenchymal cells or, possibly, vascular stem cells[12,13]. Expression of the receptor tyrosine kinase PDGFRβ in mesenchymal precursor cells, pericytes and vSMCs is required for mural-cell proliferation, guided migration and incorporation into the vessel wall. The expression, matrix-binding and correct spatial presentation of PDGFB are indispensable in mice[12,111], although PDGFD can also bind and activate PDGFRβ (FIG. 7). It has recently been suggested that PDGFB and PDGFRβ also have a role in the differentiation of haemangioblasts[112].

Neural crest cells
Ectodermal cells that delaminate from the neural tube in vertebrate embryos, migrate to various locations and contribute to different body structures such as the peripheral nervous system, bone and cartilage, skeletal and smooth muscle, or pigment cells in the skin (melanocytes).

Vascular stem cells
Stem cells that can differentiate into endothelial or mural cells in the blood vessel wall.

Haemangioblasts
Precursor cells that can differentiate into endothelial and haematopoietic cells.
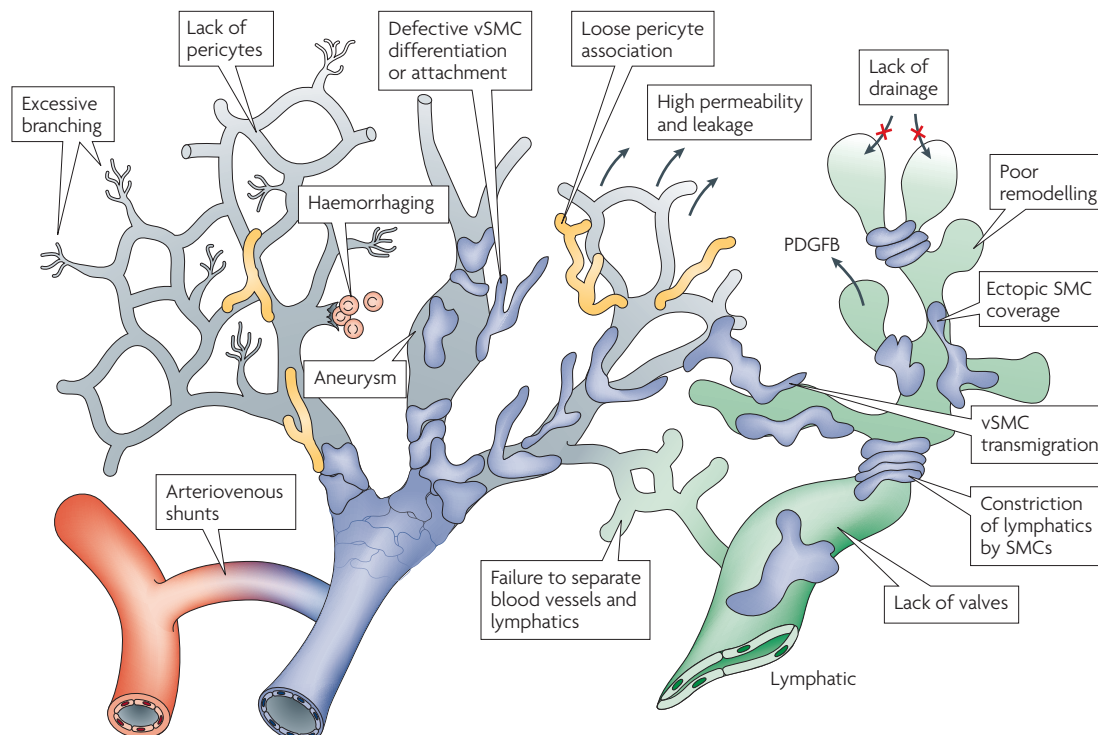
Figure 7 | **Mural cell and lymphatic defects in mutant mice and human patients.** Loss of pericytes (yellow) and vascular smooth-muscle cells (vSMCs; blue) as observed in platelet-derived growth factor B (*Pdgfb*) mutants[12,148] leads to poorly covered vessels with excessive endothelial sprouting, microaneurysms, leakage and haemorrhaging. Correct spatial presentation of PDGFB on the endothelial cell surface of blood vessels is essential for the association of pericytes and vSMCs[149]. Similarly, loss of the transmembrane protein ephrin-B2 from mural cells leads to loose irregular coverage, blood-vessel defects and ectopic smooth-muscle cell (SMC) coverage of lymphatic capillaries[105]. Ectopic PDGFB secretion, abnormal SMC association, malformation of lymphatics (green) and lack of valves compromise lymphatic function in mice that have mutations in the forkhead box transcription factor FOXC2 and in human patients with lymphoedema distichiasis[108]. Anastomoses between arteries and veins (arteriovenous shunts) or blood vessels and lymphatics have been observed in some genetic mouse models and human patients. Although the exact reasons for shunt formation are unknown, they probably involve compromised growth, differentiation and/or gene-expression programmes.

PDGFRβ function may involve cooperation with a family of G-protein coupled receptors (S1P$_{1-5}$) that bind to sphingosine-1-phosphate (S1P; also known as MBTPS1), a sphingolipid secreted by endothelial cells[113,114]. Gene-targeting experiments indicate that S1P$_1$ (also known as EDG1), S1P$_2$ and S1P$_3$ have important and partially redundant roles during vascular morphogenesis, and that the numbers of pericytes and vSMCs are compromised in S1P$_1$-deficient mice[115,116]. Tissue-specific gene-targeting experiments show that the main role of S1P$_1$ is confined to the endothelium and involves the trafficking of the cell-adhesion molecule N-cadherin to endothelial–mural-cell contact zones[117,118] (FIG. 3). Endothelial N-cadherin is indeed essential for cardiovascular development, embryonic survival and normal expression of the related adhesion molecule, vascular endothelial cadherin[119].

TGFβ1 triggers vSMC differentiation. The differentiation of progenitor cells into pericytes and vSMCs is promoted by transforming growth factor-β1 (TGFβ1)[12,13,120]. In vSMCs, TGFβ1 signalling cooperates with the myogenic transcriptional machinery, serum-response factor (SRF) and its cofactors myocardin, the cysteine-rich LIM-only proteins CRP1 and CRP2, and the zinc-finger homeobox protein δEF1 (also known as ZEB1)[121–124]. The TGFβ1 pathway has also been implicated in endothelial development. Severe vascular phenotypes have been observed in knockout mice that lack TGFβ1 (*Tgfb1*), one of the three receptors activin-receptor-like kinase-1 (*Alk1*), ALK5 (*Alk5*), TGFβ receptor-2 (*Tgfbr2*), or endoglin (*Eng*), or the downstream effector SMAD5 (REFS 12,13). Mutations in the human *ALK1* or *ENG* genes cause hereditary haemorrhagic telangiectasia, a disease that is characterized by capillary dilation, arteriovenous malformations and local bleeding[121–126].

Angiopoietins and Tie receptors. The complex interactions between the endothelium and surrounding perivascular cells are highlighted by TIE2 (TEK), a tyrosine kinase receptor that is expressed by ECs, and its ANG ligands[127,128]. Genetic experiments in mice have established that signalling by ANG1 (which is expressed by perivascular cells) and TIE2 promotes angiogenesis and pericyte association to the endothelium, reduces vascular leakage and has anti-inflammatory activity[12,128–130].

ANG2, which is produced and stored by ECs for rapid release, functions as an antagonist of ANG1 and promotes inflammatory responses[130,131]. However, ANG2 can also promote angiogenesis depending on the tissue and context[130]. Further biological roles of the angiopoietins include the regulation of EC migration, endothelial tube formation, tumour angiogenesis, haematopoiesis[130,132–135] and lymphangiogenesis (see above).

Tie1-knockout mice die during late embryogenesis and perinatally, displaying haemorrhaging and oedema[128,130]. Although it remains unclear which ligands can bind and activate TIE1, it has been recently shown that the TIE1 receptor can interact with TIE2 and signal in a heterodimeric complex[136].

Conclusions

Research in recent years has dramatically improved our understanding of the angiogenic and lymphangiogenic gene-expression and cell-differentiation programmes. New molecular regulators have been identified and are studied in increasingly sophisticated animal models such as transgenic fish or tissue-specific gene-knockout mice. Better imaging techniques allow the characterization of phenotypes at cellular or subcellular resolution as well as dynamic studies in live animals. These and other advances have made vascular morphogenesis much more accessible to research. Although we still know little about some fundamental processes, such as the formation of vascular lumens, the differentiation of venous and lymphatic valves, the interactions between endothelial sprouts, or the dynamic regulation of pericyte–endothelial contacts, progress in other areas (such as endothelial sprouting or lymphangiogenesis) is breathtaking.

One surprising discovery has been that many signalling pathways are shared between the nervous system and the vasculature. For example, VEGFA and VEGFC are not only important growth factors for the ECs but also function on neural cells[137,138]. Moreover, many molecular players, such as VEGFs, angiopoietins, ephrins and neuropilins, are involved in the morphogenesis of blood vessels and lymphatic vessels. Given that the inhibition of the angiogenic growth of blood vessels is already used in cancer therapy, simultaneous interference with the growth of both endothelial networks may prove to be increasingly powerful by inhibiting metastatic tumour-cell entry into blood and lymphatic vessels. Similarly, pro-angiogenic therapy (for example, in the treatment of chronic leg ulcers) may benefit from the simultaneous growth of new blood and lymphatic vessels.

In any case, thorough knowledge of the relevant molecular players and their functional roles in the growth, patterning and differentiation of endothelial networks will be of great importance. Fortunately, we can expect that our knowledge in these areas will continue to grow with the rapid pace seen during the past few years.

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