Therapeutic angiogenesis, which entails the induction of new blood vessels by the delivery of angiogenic growth factors, is a highly attractive approach to the treatment of ischemic diseases. However, it is becoming increasingly clear that this is not easily achieved, as the effects of angiogenic growth factors can differ markedly depending on the timing of their expression, on the shape of the concentration gradients they form in vivo, and the interactions between endothelial cells and pericytes they induce. In fact, the same dose of vascular endothelial growth factor can induce stable, nonleaky, pericyte-covered normal capillaries or aberrant vascular structures that develop into hemangiomas. This difference in outcome can be due solely to the spatial characteristics of the delivery method. If delivery allows a homogeneous spatial distribution of VEGF in the microenvironment around each producing cell, angiogenesis can be therapeutic, whereas if the total dose is the average of diverse spatial levels, aberrant angiogenesis cannot be avoided. To achieve therapeutic angiogenesis, a means of regulating the microenvironmental levels of angiogenic factors will be critical to the generation of effective new treatment strategies.

The Angiogenic Microenvironment
The first vascular structures in the embryo are assembled by in situ differentiation of scattered progenitor cells into blood islands, which give rise to the primitive vascular plexus by the process of vasculogenesis. However, the further vascularization that accompanies growth and development occurs through angiogenesis, which is the sprouting of microvascular capillaries from pre-existing postcapillary venules. After morphogenesis and growth are complete, the vasculature is mostly quiescent in the adult. However, it retains the ability to initiate the angiogenic program at any time, such as in physiologic events like the female reproductive cycle, in pathologic processes such as tumor growth, or in the tissue repair that accompanies wound healing and in response to ischemia. The generation of new capillaries from neighboring microvasculature by angiogenesis can be represented as a two-step process: 1) tube formation, in which endothelial cells respond to gradients of angiogenic factors, proliferate, and migrate towards areas where increased blood flow is needed; and 2) vascular maturation, in which pericytes are recruited to proliferating endothelium and induce quiescence and stabilization of the new capillaries through cell-cell contact and paracrine factors. The formation of a new vascular network with normal morphology and physiologic function is critically dependent on the harmonious interaction both in space and time of different cell types and secreted factors [1]. This process is not simply a result of global overexpression or inhibition of certain factors alone, but requires the spatially and temporally restricted organization of their availability at the microenvironmental level. In this article, we review the current understanding of how the growth of normal or pathologic blood vessels is determined by microenvironmental factors and what lessons can be
learned to design more physiologic strategies to achieve therapeutic angiogenesis for the treatment of ischemia.

**Vascular endothelial growth factor and its gradients**

When arterial insufficiency causes ischemia in the downstream vascular bed, the parenchymal cells respond to the resulting hypoxia by rapidly switching on a co-ordinated pattern of gene expression driven by the transcription factor hypoxia-inducible factor-1 (HIF-1). Active HIF-1 is composed of two subunits (α and β). Both subunits are constantly produced, but HIF-1α is continuously hydroxylated in the presence of oxygen and targeted for destruction in the proteosome by ubiquitination. In hypoxia, however, this degradation ceases, the active dimer is stabilized, and HIF-1 directs the transcription of a variety of genes involved in energy metabolism, oxygen transport, and angiogenesis. Among these are vascular endothelial growth factor (VEGF)-A, its receptor Flt-1, and angiopoietin-2 (Ang-2), which initiate the sprouting of new capillaries [2]. A variety of growth factors have been found to have angiogenic properties [3], but VEGF remains the fundamental angiogenic factor, as evidenced by the fact that even variations in its levels of expression as small as a 50% reduction [4,5] or a two- to threefold increase [6] preclude normal vascular development and cause embryonic lethality. The exquisite potency and specificity of VEGF as an endothelial mitogen has made it a major target for clinical trials of therapeutic angiogenesis.

Recent studies have shown that the spatial distribution of VEGF within the tissue microenvironment has a fundamental role in regulating normal capillary growth and arteriovenous patterning. The VEGF molecule exists as at least three major isoforms of 120, 164, and 188 amino acids in the mouse (and 121, 165, and 189 in man), which arise from the same mRNA by alternative splicing and which differ in the size of their heparin-binding domain at the carboxy-terminus. The shortest is completely soluble whereas the larger ones are less soluble due to increased binding to the extracellular matrix [7]. Transgenic mice selectively expressing only one of the three isoforms (VEGF120/120, VEGF164/164, and VEGF188/188) provide ideal tools for understanding how VEGF’s microenvironmental distribution affects angiogenesis. VEGF120/120 mice exhibit no embryonic lethality, but immediately after birth display a striking defect in growth of myocardial capillaries, which are tortuous and dilated [8]. In addition, impaired angiogenesis caused the growing heart muscle in these mice to become rapidly and progressively ischemic, with death occurring within 12 days due to cardiac failure. Most striking was the finding that angiogenesis was deficient and aberrant despite the fact that total VEGF expression levels were similar to wild-type levels, except that it was comprised only of VEGF120 rather than a mixture of the three isoforms [8].

Vessel development can be studied very conveniently in the murine retina because its vascularization begins immediately after birth and proceeds centrifugally from the optical disc to the periphery in a highly stereotypical fashion. In addition, its growth is primarily two-dimensional, which makes the retinal capillary plexus readily amenable to whole-mount imaging. Analyses of retinal vascularization revealed that, whereas VEGF164/164 mice had normal vessel growth and patterning, matrix-bound VEGF188 induced increased capillary formation and defective arteriolar development. In contrast, soluble VEGF120 caused severely impaired outgrowth of all vessel types, the formation of larger capillaries, and dilatation and tortuosity of hyaloid vessels [9]. Ruhberg et al. [10] have shown that the basic anomaly in VEGF120/120 mice is a widespread patterning defect that results in vascular networks with reduced branching and increased capillary diameter, which is not caused by loss of signaling through the VEGF164-specific receptor neuropilin-1. Instead, this was due to a preferential integration of endothelial cells into existing vessels rather than into additional sprouts and was correlated with a disruption of the extracellular VEGF gradients in the mutant mice [10]. Conversely, VEGF188/188 mice showed opposite defects, with ectopic branching and reduced capillary diameter [10]. Remarkably, VEGF120/188 mice, which expressed both the 120 and 188 isoforms but no VEGF164, developed completely normal vasculature just like the VEGF164/164 mice, which only have VEGF164 [10].

Gerhardt et al. [11] have elegantly characterized the role of VEGF distribution and gradients in precisely directing capillary formation in retinal vascularization. The first endothelial cell of the growing sprout is a specialized tip cell, which has no lumen, senses a VEGF gradient by extending several thin filopodial processes, and migrates towards its source without dividing. In contrast, proliferation only occurs in the subsequent endothelial cells of the stalk, which also form the primitive lumen of the sprout. Interestingly, whereas tip cells respond to the gradient of VEGF distribution, stalk cell proliferation is regulated by its absolute concentration [11].

Collectively, this body of data yields an overall model in which the balanced production of soluble and heparin-binding isoforms determines the shape of the VEGF gradient in the microenvironment around the secreting cells. This balance controls whether proliferating capillaries grow into polarized branches by directed tip cell migration or enlarge circumferentially by non-directional cell proliferation.

**Pericyte recruitment**

Although the regulation of endothelial cell assembly into perfused vessels has received much attention in angiogenesis, this is only half of the picture. For the generation of stable and functional capillaries, it is necessary that the nascent endothelial tubes are invested with pericytes and intimate communication is established between the two cell types. Pericytes reside on microvascular capillaries as
individual cells and contact several endothelial cells with their long, thin processes, whereas vascular smooth muscle cells (vSMC) coat arteries and veins as a continuous layer. Both cell types are collectively referred to as mural cells and have been suggested to represent different phenotypes of the same lineage with separate functions. Association with mural cells renders vessels independent of continued VEGF expression, whereas those lacking pericytes regress following its withdrawal [12,13]. In diabetic retinopathy, hypoxia leads to an excessive production of VEGF in the retina and the formation of pericyte-poor vessels that bleed easily and can ultimately lead to blindness [14–16]. Pericytes have also been proposed to control vessel sprouting and branching [17]. In summary, pericytes are fundamental to the maintenance of vascular homeostasis and regulation of angiogenesis as they sit at the interface between the parenchyma and the vascular compartments and provide paracrine signaling to endothelium, regulating its proliferation and differentiation [18].

During capillary formation, the migrating tip cell produces platelet-derived growth factor-B (PDGF-B) and recruits pericytes, which express PDGF receptor-β (PDGFR-β) to the sprouting endothelium [11•]. Indeed, mice in which the pdgf-b gene was deleted specifically in endothelial cells (pdgf-b<sub>−/−</sub>), although viable, display a diffuse and variable defect in pericyte recruitment, with pericyte numbers reduced by as much as 90% of normal, and evidence of multigorgan microvascular defects reminiscent of diabetic microangiopathy [19]. In the complete absence of the PDGF-B or PDGFR-β genes, pericyte formation is not affected, but their recruitment to nascent vasculature is severely impaired and results in perinatal mortality due to vessel instability and formation of microaneurysms and hemorrhage [20,21]. Interestingly, analysis of a series of mutant mice, in which the PDGFR-β is mutated to prevent binding of specific signal transduction components (hypomorphic receptor function), has revealed that no individual signaling pathway downstream of PDGFR-β is crucial for pericyte development, but rather their total number is modulated by both the amount of receptor expressed [15,22] and the number of active downstream pathways [22].

Like the angiogenic response of endothelium to VEGF, pericyte recruitment is also a process that is highly dependent on PDGF-B microenvironmental distribution. The PDGF-B molecule contains a positively charged amino acid stretch at its carboxy-terminus, which mediates its retention on the cell surface and in the extracellular matrix, like the long isoforms of VEGF. Mutant mice, in which this retention motif was deleted from the endogenous PDGF-B gene (pdgf-B<sub>ΔN</sub>) and in which PDGF-B diffuses freely away from the producing cells, develop only about 50% of the normal amount of pericytes. Especially important is the finding that the pericytes in these mice are abnormally associated with capillaries and are apparently detached from endothelium, sending their processes away from the vessels [23]. These defects caused severe retinal deterioration, proteinuria, and glomerulosclerosis [23]. In comparison, the pdgf-B<sub>ΔN</sub> mice in which the pdgf-b gene was deleted specifically in endothelial cells displayed a milder retinopathy despite a much more extensive reduction in pericyte number of up to 90% [14]. However, the few remaining pericytes in pdgf-b<sub>ΔN</sub> mice were normally associated with the abluminal endothelial surface, suggesting that retention of PDGF-B and its formation of steep gradients in the pericellular space are crucial for appropriate pericyte recruitment and establishment of normal pericyte/endothelial cell contact, without which pericyte function is defective. This concept is further reinforced by experiments with tumors grown in pdgf-b<sub>ΔN</sub> mice [24•]. Their vessels had fewer and aberrantly associated pericytes compared with the same tumor grown in wild-type mice, and the exogenous expression of normal PDGF-B by the tumor cells was able to rescue the deficit in pericyte number but not the defect of pericyte integration into the capillary wall [24•].

**Endothelial-pericyte cross-talk**

The crucial role of pericyte-endothelial communication is clear from the finding that pericyte-deficient vessels in PDGF-B and PDGFR-β knockout mice exhibit endothelial cell hyperplasia, variable diameter, abundant microaneurysms, abnormal endothelial ultrastructure, and increased permeability, leading to perinatal lethality [25]. In addition, when pericyte recruitment is prevented by inhibiting PDGFR-β kinase activity, the antiangiogenic activity of endothelial VEGFR inhibition is increased in a model of insulinomas [26]. Conversely, co-implantation of the mesenchymal precursor cells 10T1/2 with endothelial cells caused tissue-engineered vascular networks to stabilize and persist for up to 1 year in vivo with functional perfusion [27].

Although the understanding of the molecular cross-talk between pericytes and endothelial cells and how they regulate each other’s physiology is still in its infancy, the signaling pathways of transforming growth factor-β (TGF-β), angiopoietins, and VEGF have been shown to play a role.

When pericytes are recruited to sprouting endothelium, both cell types express TGF-β1 and its receptor, but signaling does not occur because the factor is inactive. Activation only occurs at the interdigitating cell-to-cell contact sites, by proteolytic cleavage of the latency-associated peptide by plasmin, and promotes vascular stabilization at the vessel wall interface. Indeed, active TGF-β1 inhibits endothelial cell proliferation and migration, stimulates mural cell differentiation, and is required for the formation of capillary structures [28].

Angiopoietins (Ang) are the ligands of the endothelium-specific tyrosine kinase receptor Tie2. Ang1 is an antagonist of Ang1 on the Tie2 receptor. It promotes the dissociation of endothelial cells from periendothelial support cells and
is, therefore, necessary for angiogenic remodeling of the vasculature. In the absence of VEGF, Ang2 leads to vascular regression, whereas in the presence of VEGF, Ang2 facilitates vascular growth [3,29]. By contrast, Ang1 is expressed by pericytes in vitro and in vivo [30] and, by activating Tie2 signaling, Ang1 facilitates the recruitment and association of mural cells with nascent vessels, acts as a survival signal for endothelial cells, and inhibits VEGF-induced vascular leakage [3].

Intriguingly, it has been recently shown that a modified recombinant form of Ang1 (Ang1*) can completely rescue the vascular defects induced by preventing pericyte recruitment in retinal vascularization by means of an antibody that blocks PDGF-β [31]. Despite the persistent absence of mural cells, Ang1* injection could restore a properly remodeled hierarchical vascular network and prevent edema and hemorrhage [31].

Nascent vasculature is critically dependent on VEGF signaling for survival and regresses if it is withdrawn, as elegantly shown in a transgenic mouse model of conditional tissue-specific VEGF expression [32]. VEGF could be reversibly induced exclusively in the heart or liver by adding or removing tetracycline in the drinking water and it could be shown that, whereas early withdrawal caused all new vessels to regress, after stabilization and maturation they had become independent, persisted after VEGF withdrawal, and actually remodeled to a more physiologic morphology through hemodynamic forces [32]. The mechanism by which maturation causes vessel independence from tissue-derived VEGF has been recently suggested to involve expression of VEGF from differentiating pericytes upon establishment of cell-to-cell contact with sprouting endothelial cells [33]. In particular, induction of VEGF expression was dependent on local TGF-β1 activation in the endothelial-mural cell interstitium and signaling through the Smad-3 pathway in pericytes, and it provided a continued survival signal to the endothelium of stabilized retinal capillaries [33].

**Inflammatory cells and matrix remodeling**

The important role of the extracellular matrix, vascular basement membrane, and the interstitial stroma in the angiogenic process has become increasingly clear in recent years [34]. The endothelial cells are embedded in the vascular basement membrane (composed mainly of laminin and collagen IV), which acts as a scaffold and needs to be degraded and remodeled in a temporally and spatially controlled manner in order to enable angiogenesis. One of the first steps entails active proteolytic enzymes that are generated locally to cleave the extracellular matrix, allowing the detachment of perivascular cells and creating a route for endothelial cell migration. The members of the matrix metalloproteinase (MMP) family are the most prominent proteolytic enzymes involved in angiogenesis, which, as a family, are able to degrade all components of the extracellular matrix. Their activity is tightly regulated at different levels, including gene expression and activation of the zymogen. Furthermore, potent endogenous inhibitors, such as the tissue inhibitors of matrix metalloproteinases (TIMPs), act to prevent uncontrolled protein degradation. Proteolytic activity is further controlled by spatial restriction, as exemplified by the membrane-bound protease MT1-MMP, which localizes its activity to the cell surface. Adding to the complexity of the picture, members of other proteinase families, such as the cathepsins, have also been shown to be critically involved in angiogenesis.

The role of the extracellular matrix, however, goes beyond that of physical barriers. It acts as a reservoir for growth factors, such as VEGF, that can be locally released on demand by active MMP-9 without increasing total VEGF levels in the tissue [35]. These extracellular sources of angiogenic growth factors, involved in the “angiogenic switch” in tumorigenesis, may provide microenvironmental gradients that spatially direct the outgrowth of angiogenic vessels. Increased vascular leakage induced by VEGF and extracellular protease activity leads to the deposition of a provisional matrix in which cryptic cell binding sites are exposed, providing specific guidance cues to migrating endothelial cells that express a different repertoire of adhesion molecules upon activation [36]. Monocytes are recruited by VEGF and, in turn, produce more proteolytic enzymes and angiogenic growth factors [37]. To hold the process in balance, proteolytic degradation of the extracellular matrix also gives rise to antiangiogenic molecules, such as angiotatin and endostatin, which are collagen-cleavage products generated by different MMPs and cathepsin L [34].

**The Microenvironment in Therapeutic Angiogenesis**

Several clinical trials in the past decade have tested the potential of fibroblast growth factor (FGF) or VEGF family members for achieving therapeutic angiogenesis in cardiac or limb ischemia by delivery of recombinant protein, naked plasmid DNA, or adenoviral vectors. Although several phase I studies have demonstrated safety, phase II studies have thus far failed to demonstrate significant clinical efficacy [38]. In particular, for the VEGF164 gene, the paradox seems to lie in an apparently very narrow therapeutic window, such that whereas low doses appear inefficacious, only slightly higher doses induce progressive growth of hemangioma-like vasculature [39,40]. Recent evidence from our laboratory indicates that this is not because VEGF has an intrinsically steep dose-response curve, but rather because the dose delivered must be controlling at the microenvironmental level [41••]. We used a retrovirally transduced primary myoblast population to drive constitutive expression of exogenous VEGF in skeletal muscle in vivo and, as already described, this high level induced the progressive growth of hemangioma-like vessels. Because retroviral vectors integrate stably in the
genome, we were able to characterize precisely a range of diverse VEGF-expressing cell populations in vitro and isolate individual clones expressing a given dose. In this way, we could manipulate the VEGF dose so that it was the same on a cell-by-cell basis. The results distinguished between total dose delivered and microenvironmental level of expression.

When the total VEGF levels resulting from heterogeneous populations of cells were reduced by diluting the cells, aberrant hemangioma-like vessels could not be avoided, no matter how little total VEGF was expressed [41••], as shown in Figure 1. The total amount of VEGF expression could be varied about 12-fold between 5 and 60 ng/10⁶ cells per day, in a manner conceptually similar to decreasing the titer of an adenoviral vector or the concentration of a plasmid in gene therapy trials, yet hemangiomas resulted.

By contrast, when the VEGF concentration was controlled in the microenvironment around each transgenic fiber, a dose-dependent threshold between normal and aberrant morphology became evident. This was achieved by selecting and implanting different clonal populations that expressed increasing total VEGF levels, but within which every myoblast produced the same amount of VEGF [41••]. As shown in Fig. 2, microenvironmental VEGF levels across a 14-fold range (from 5 to 70 ng/10⁶ cells per day) induced the growth of stable, uniformly sized capillaries that were associated with pericytes, were not leaky, and eventually became independent of continued VEGF expression [41••]. By contrast, only when a microenvironmental VEGF level of approximately 100 ng/10⁶ cells per day or above was reached was aberrant angiogenesis induced, which inevitably eventually resulted in the growth of hemangiomas.

In summary, whereas a total dose of VEGF as low as 5 ng/10⁶ cells per day unavoidably caused hemangioma growth when produced as an average value by nonhomogeneous polyclonal cells (Fig. 1), a dose 14 times higher (70 ng/10⁶ cells per day) produced by a homogeneous population of cells (a clone) reproducibly gave rise to the growth of normal stable capillaries (Fig. 2). Thus, doses of VEGF cannot be averaged even across small distances, but must be controlled at a microenvironmental level.

This concept is schematically represented in Figure 3. When the VEGF gene is delivered to ischemic muscle, different fibers will be transduced with a different number of copies of the vector, therefore creating a mosaic of expression levels, ranging from low and inefficacious to high and dangerous, as represented by the wide distribution curve in the upper panel. As long as even a few of these expression levels are above the threshold, hemangioma formation will follow, because VEGF protein remains tightly localized around the producing cell in vivo and, therefore, even rare “hotspots” of expression cannot be averaged with neighboring areas. Manipulating the total dose, without affecting its distribution, is equivalent to shifting the whole curve without altering its shape. It is clear that, in order to reproducibly avoid expression above the threshold level, the total dose delivered needs to be such that the majority of transduced fibers express rather low, and probably inefficacious, levels (Fig. 3A). On the other hand, controlling the microenvironmental distribution of VEGF is equivalent to altering the shape of the curve, reducing its breadth, and allowing the totality of expression levels produced in vivo to be in the high range of the therapeutic window, and yet remain below the threshold that causes hemangiomas (Fig. 3B). In this situation, both efficacy and safety can be achieved.
This conceptual difference may partly explain the apparent lack of efficacy, at safe doses, for VEGF gene delivery in clinical trials of therapeutic angiogenesis, in which only the total dose of vector administered could be controlled by varying the viral titer or the plasmid concentration, but not its microenvironmental distribution in the tissue.

Therapeutic perspectives

The significant knowledge acquired in the past few years about the fine regulation of blood vessel growth by factor gradients and the molecular cross-talk between different cell types has allowed the design of novel therapeutic strategies that take into account the complexity of the angiogenic microenvironment. They can be schematically categorized in three conceptual families: 1) control of the microenvironmental distribution of a single angiogenic factor; 2) modulation of the effects of one angiogenic factor by the co-delivery of a maturation factor; and 3) induction of a complex angiogenic response by the delivery of a “master-regulator.”

Controlling the microenvironmental dose is easier to advocate than to achieve. The genetic manipulation of progenitor cells ex vivo and their selection before reimplantation, although currently cumbersome for clinical application, has the potential to precisely control the microenvironmental distribution of VEGF and its dose-dependent effects [41••]. Alternatively, the controlled release of recombinant VEGF protein, achieved through its incorporation into slowly degrading polymer matrices, may allow a similar prolonged delivery of homogeneous concentrations of growth factor in vivo [42]. Yet another solution may be the delivery of a minigene allowing the simultaneous expression of all three major VEGF isoforms, which has been reported to be 100-fold more potent than delivering each individual isoform separately.

Figure 2. If the microenvironmental distribution of expression levels is controlled, a dose-dependent threshold between normal and aberrant angiogenesis becomes apparent and the same or higher total doses reproducibly induce normal capillaries. (VEGF—vascular endothelial growth factor.) (Adapted from Ozawa et al. [41••]; with permission.)

Figure 3. Conceptual representation of the clinical implications of the data shown in Figures 1 and 2. A, If only total dose delivered can be controlled, but not microenvironmental distribution, therapeutic efficacy will not be achieved in order to avoid rare areas of expression above the threshold, which cause hemangioma formation (see broad distribution of vascular endothelial growth factor levels). B, When homogeneously distributed doses can be delivered, the microenvironmental distribution can be tightly controlled and efficacy achieved without risk of deleterious effects (see narrow distribution of vascular endothelial growth factor levels). (VEGF—vascular endothelial growth factor.)
isoform alone in restoring blood flow to the ischemic mouse hind-limb [43].

Exogenous administration of angiopoietin-1 has been shown to stabilize new vessels and counteract VEGF-induced vascular leakage and edema [44], making it an attractive candidate for co-delivery with VEGF. Given the fundamental role of pericytes in vascular maturation, both through paracrine signaling and cell-to-cell contact, it seems logical that stimulating pericycle recruitment with PDGF-BB could modulate the deleterious effects of VEGF delivery. The co-delivery of PDGF-BB has been recently found to induce a significant synergistic angiogenic response with FGF-2, but not VEGF, in the mouse cornea [45]. The lack of effect on VEGF-induced angiogenesis may, in part, be specific to the avascular microenvironment of the cornea, as the co-delivery of VEGF and PDGF-BB subcutaneously and in the ischemic hindlimb has been reported to cause a significant increase in the maturation index of the newly formed vessels [46]. A third strategy consists of inducing a coordinated angiogenic response by administering a “master-regulator,” which could induce the expression of a host of molecules (not just one or two) through an endogenous transcriptional pathway. Overexpression of a constitutively active mutant of HIF-1α caused robust angiogenesis, including nonleaky vessels, which retained the physiologic ability to increase their permeability in response to inflammatory stimuli [47] and increased blood flow in the ischemic hindlimb [48]. Targeting the same pathway, the PR39 peptide, which is naturally produced by macrophages, has been found to inhibit HIF-1α degradation and to improve coronary blood flow when delivered by gene therapy [49]. Also, the delivery of a specifically engineered zinc-finger transcription factor has been shown to be capable of upregulating the transcription of the endogenous VEGF gene, inducing the growth of nonhyperpermeable new vasculature, and accelerating wound healing [50].

Conclusions
The growth of blood vessels involves a complex series of molecular and cellular interactions, among which a fundamental role is played by the spatial distribution and temporal expression of different signaling and matrix molecules within the angiogenic microenvironment. This complexity has only begun to be appreciated recently. This understanding of the fine regulation of vascular growth now provides the fundamental knowledge that should lead to the design of novel and more efficacious biology-oriented therapeutic strategies for the treatment of ischemic diseases.

Acknowledgments
This work was supported by an American Heart Association Scientist Development Grant 0430312N to AB, by a grant from the Deutsche Forschungsgemeinschaft DE 740/1-1 to GvD (0430312N), and by NIH grants AG009521, HL065572, HD018179, AG020961, AG024987 and the Baxter Foundation to HMB.

References and Recommended Reading
Papers of particular interest, published recently, have been highlighted as:
• Of importance
• Of major importance
This paper shows that diffusible and heparin-binding VEGF isoforms control the morphogenesis of new capillaries and induce circumferential growth of branch formation, respectively, through the shape of the concentration gradients they form in the tissue. A balance between the isoforms is necessary for physiologic capillary morphogenesis.
This paper shows that VEGF concentration gradients are sensed by a specialized tip cell on endothelial sprouts, which responds by migrating towards the source. On the other hand, proliferation occurs only in the following stalk cells and is regulated by VEGF concentration, not its gradient. Migration and proliferation of the capillary sprout are regulated independently by two different qualities of VEGF distribution in the microenvironment, sensed by distinct endothelial cell populations.
24. •Abramsson A, Lindblom P, Betsholtz C: Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. J Clin Invest 2003, 112:1142–1151. This paper shows that removal of the cell-surface retention motif from the PDGF-B molecule abrogates the steep gradient it normally forms around endothelium and this disrupts pericyte recruitment. Delivery of exogenous wild-type PDGF-B can increase the number of pericytes but cannot restore their proper investment in the vessel wall, as it is not produced by the endothelial cells.