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Review

Between Molecules and Morphology

Extracellular Matrix and Creation of Vascular Form

Robert B. Vernon and E. Helene Sage

From the Department of Biological Structure, University of Washington, Seattle, Washington

The generation of new microvasculature from extant vessels (angiogenesis) is intimately associated with a variety of biological processes that include growth and development, the repair of wounds, increases in body mass, the menstrual cycle, and pregnancy. Angiogenesis begins when endothelial cells (ECs) of the parent vessel (typically a capillary or postcapillary venule) detach from the vascular wall, degrade and penetrate the basal lamina that invests them, and invade the surrounding interstitial extracellular matrix (ECM) as a knoblike or cone-shaped vascular bud or sprout. Subsequent stages of angiogenesis involve increases in the length of individual sprouts, the formation of lumens, and the anastomosis of adjacent sprouts to form vascular loops and networks.[1–3] These morphogenetic processes require ECs to exhibit a special set of complex behaviors (sometimes referred to as an angiogenic phenotype[4]) that include migration, proliferation, intercellular alignment and adhesion, and generation of a patent lumen.

Abnormal development of blood vessels characterizes a variety of disorders that include rheumatoid arthritis, diabetic retinopathy, hemangiomas, psoriasis, and the growth and metastasis of tumors; consequently, understanding the mechanisms that regulate vascular growth is now recognized as an important contribution to the treatment and resolution of these pathologies. From the appreciation that genes regulate cellular behavior has arisen the proposition that angiogenesis is orchestrated by a program or cascade of spatially and temporally controlled expression of EC gene products. Evidence indicates that the expression of specific gene products by ECs is stimulated or inhibited by a variety of extracellular growth factors that are associated with target tissues, organs, and tumors.[5]

The idea that angiogenic behaviors among ECs are controlled by internal genetic programs that, in turn, are influenced by external regulatory factors has been an important paradigm for investigation; a survey *via* the Medline database of angiogenesis literature revealed that, of 882 papers published from 1983 to 1991, 92% dealt mainly or exclusively with factors that induce angiogenesis.[6] Without a doubt, studies of the expression and regulation of genes relevant to angiogenesis are crucial to the identification of molecular targets for the clinical control of vascular growth; however, such studies alone cannot fully explain the specific mechanisms that create vascular form. The regulation of gene products is quantitative, ie, synthesis of individual proteins can be started, stopped, or varied in amount. The formidable and unsolved problem of vascular morphogenesis is to determine the means by which the quantitative modulation of specific proteins by the angiogenic programs of individual ECs generates spatial information that directs the cells to form complex structures, ie, multicellular tubes organized into extensive anastomotic networks (Figure 1). It is reasonable to assume that the nucleus, cytoplasm, and cell surface of ECs are sites of action for gene products relevant to vascular morphogenesis. However, in addition to these cellular compartments, we propose that the ECM might play a particularly important role in the translation of unidimensional gene regulation to three-dimensional vascular structure.

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Address reprint requests to Dr. Robert B. Vernon, Department of Biological Structure, SM-20, University of Washington, Seattle, WA 98195.

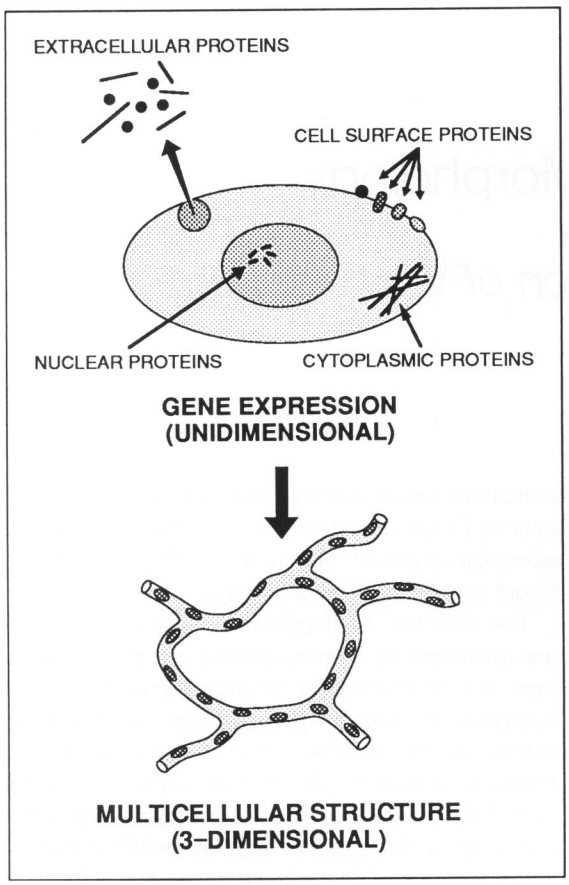

Figure 1. *The problem of vascular morphogenesis. ECs that initiate angiogenesis exhibit changes in the synthesis of specific proteins of the nucleus, cytoplasm, cell surface, and extracellular milieu. How is this quantitative, unidimensional information translated into a three-dimensional structure, ie, a network of multicellular tubes?*

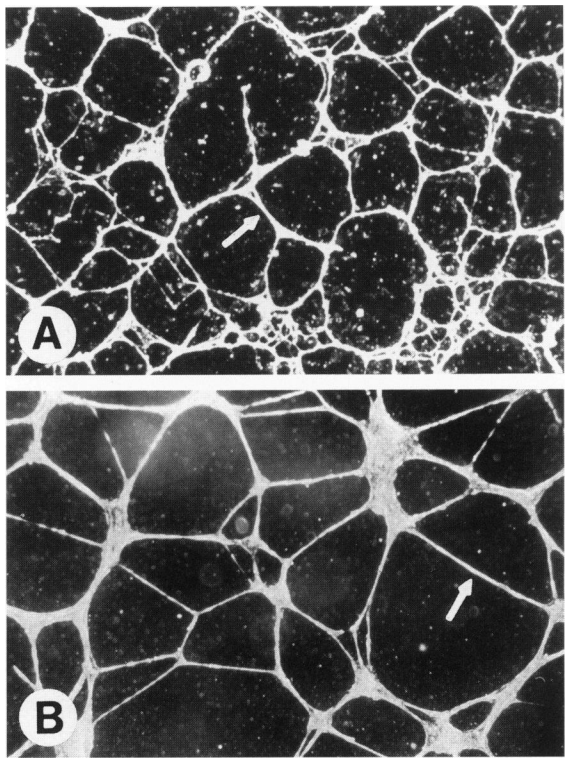

Figure 2. *Isolated ECs organize into capillary-like patterns* in vitro. **A:** *An example of spontaneous angiogenesis. Ten days after becoming confluent on tissue culture plastic, a monolayer (dark background) of bovine aortic ECs generates cords of cells (eg,* arrow*) arranged in a planar network that lies parallel to the surface of the culture plate.* **B:** *ECs cultured in contact with ECM form networks rapidly. In this example, subconfluent bovine aortic ECs have organized into a planar network of cords* (arrow) *within 24 hours of plating on a layer of Matrigel.* **A** *(×9) and* **B** *(×24) are viewed by darkfield illumination.*

Microvasculature in a Dish

The process of vascular growth is difficult to monitor and to manipulate experimentally *in vivo*; therefore, investigators have devised developmental models in which ECs form multicellular cords or tubelike structures *in vitro* that resemble microvascular sprouts or networks. In one approach, ECs are induced to sprout directly from explanted segments of macrovasculature (eg, rat aortas sectioned into rings[7–10]); however, the majority of models generate capillary—like structures from monotypic cultures of macrovascular or microvascular ECs. Culture systems can be simple: networks of cellular cords will arise from confluent monolayers of ECs that are grown on unmodified tissue culture plastic,[11–16] a phenomenon sometimes referred to as spontaneous angiogenesis *in vitro* (Figure 2A). Use of spontaneous angiogenesis as a model is somewhat inconvenient because it occurs only rarely among cultured ECs. Moreover, spontaneous development of cellular networks can require periods of culture as long as 6 weeks.[11] Angiogenesis-like behaviors are expressed more rapidly and among a greater variety of ECs in culture systems where the ECs are placed in contact with ECM. It is noteworthy that a variety of types of ECM (eg, clotted fibrin, type I collagen, basement membrane matrix, or serum fibronectin) in different forms (such as thin films or thicker gels) induce morphogenetic activity among ECs *in vitro*.[7,8,10,13,17–19]

The influence of ECM on the generation of vascular patterns *in vitro* is perhaps most clearly illustrated when ECs are cultured on top of layers of gelled basement membrane matrix (Matrigel); within 24 to 72 hours the cells organize into an extensive network that resembles a capillary mesh (Figure 2B).[17,20–23] We have shown that the orientative behaviors of ECs on Matrigel depend upon the reorganization of a portion of the Matrigel into a network of narrow tracks or cables; ECs that contact the matrical network assume elongate, bipolar shapes, migrate along the aligned matrix, and with time, colonize it to form a

corresponding network of cellular cords.[21] Recently, we found that the formation of networks of ECs in the absence of experimentally supplied layers of ECM (ie, during spontaneous angiogenesis *in vitro*) is also mediated *via* the association of the ECs with organized ECM. In this culture system, the confluent monolayer of flattened, polygonal ECs generates an underlying network of cables from endogenous ECM (primarily type I collagen) polymerized *in situ*. ECs of the monolayer that contact the collagenous network assume spindle shapes, align, and associate with one another to form cellular cords.[16] Portions of the network of ECs, supported by their scaffold of collagen, eventually become elevated above the monolayer of ECs. It is interesting that ECM *in vitro* becomes organized differently according to cellular density (on Matrigel, subconfluent ECs will form networks whereas the spontaneous model requires a confluent monolayer before the development of networks) and specific macromolecules (Matrigel contains laminin, type IV collagen, and fibronectin but lacks type I collagen). The variable circumstances under which networks form underscore the mechanical nature of the process, which involves the response of ECM to tension generated by a planar field of cells.

Traction, Tension, and Tessellation in Vitro

Fibroblasts, endothelial cells, and a variety of other cell types distort malleable substrates *in vitro* as they move over them by a process referred to as traction.[21,24,25] The phenomenon of traction can be visualized by the culturing of cells on thin films of polymerized silicone; the pull of each cell on the sheet of rubber generates tension wrinkles in the sheet that emanate from beneath each cell body.[21,26] Concomitantly, the cells compact the rubber that lies directly beneath them into a pleated array of compression folds.[21,26] Cells exert traction on ECM in a similar manner, and it is *via* traction that monolayers of subconfluent ECs organize a planar substrate of ECM *in vitro* (eg, gels of Matrigel or type I collagen) into a complex pattern[21] (Figure 3, A–E). Each EC (or aggregate of ECs) acts as a traction center by its continual pulling of ECM toward itself. With time, the traction centers generate radiating traction fields of strain within vicinal ECM. Where adjacent traction fields overlap, tension within the ECM is enhanced (the two-center effect),[26] an effect causing fibers of ECM to align into narrow tracks that connect the traction centers.[21,24,25] A planar field of traction centers will become connected by multiple two-center effects that form a tessellated (ie, network-like) pattern within the layer of ECM. When the layer of ECM is highly malleable, the stresses generated by the centripetal movement of ECM to traction centers will perforate the sheet of ECM and reorganize it into a web of cables[16,21,25] (Figure 3F). Our evidence indicates that it is *via* the conversion of ECM from sheets to webs that networks of type I collagen are generated by confluent monolayers of ECs during spontaneous angiogenesis *in vitro*.[16] Planar networks of ECs typically arise *in vitro* in intimate association with ECM[11–13,27,28]; therefore, it is likely that traction-mediated tessellation of ECM is typical of models of vascular development that generate flat networks of ECs.

Limitations of Planar Angiogenesis in Vitro

The behavior of ECs in planar culture systems has been referred to as angiogenesis *in vitro*, largely on the basis of the network-like organization of the cells. It is apparent, however, that planar models exhibit characteristics that are atypical of angiogenic neovascularization *in vivo*. For example, planar networks of cells form by tessellation that is simultaneous (more or less) throughout a field of pre-positioned ECs, a process that differs from the growth of angiogenic sprouts that arborize by multiple levels of branching *in vivo*. ECs within planar networks frequently enfold the supportive scaffold of ECM and thereby assume tubular shapes that in some cases resemble capillaries.[27,28] The morphogenesis of vascular lumens *in vivo* is poorly understood. Therefore, it has been proposed that cords of ECM might mediate capillary tubulogenesis by their acting as mandrels around which the endothelial cells could wrap,[11] although matrical mandrels have not been found *in vivo*. Moreover, the clearance of mandrel material from the centers of EC tubes *in vitro*, which is necessary to establish a functional lumen, is uncommon. A better simulation of angiogenesis with respect to invasion of ECM and formation of lumens is achieved in cultures in which monolayers of ECs penetrate thick substrates of gelled type I collagen in response to phorbol esters or polypeptide growth factors.[29,30] The ECs enter the collagen as invaginations from the surface of the cellular monolayer and form branched tubes with patent, fluid-filled lumens, a process that resembles tubulogenesis during the early stages of sprout formation *in vivo*, in which the lumen of the parent vessel is extended into the sprout as an outpocketing or system of channels.[1,6]

Contact with malleable ECM *in vitro* causes a variety of cell types, including ECs, to exhibit characteristics of differentiation that include decreased proliferation, elevated expression of cell-specific gene products, and appropriate responses to molecular signals. Although it is likely that ECs cultured on ECM increase the expression of molecules contributing to traction-mediated tessellation, the view that the tessellation event itself is an indicator of vascular differentiation should be approached with caution. We observe that non-EC types that include dermal fibroblasts, aortic smooth muscle cells, Leydig cells, and TM-3 cells (a murine Leydig cell line) generate networks of cells and ECM on Matrigel by traction.[21,31] Moreover, planar multicellular webs or networks formed by retinal pigmented epithelial cells,[32] osteoblast-like cells,[33] kidney epithelial cells,[34] a hepatocyte cell line,[35] and cells from enteric ganglia,[36] grown in contact with Matrigel or layers of collagen, have morphologies indicative of a traction-mediated mechanism of pattern formation. The fact that a variety of cell types generate networks *in vivo* is not surprising, as the phenomenon depends upon the mechanical properties of an acellular ECM and a behavior (traction) common to many cells.

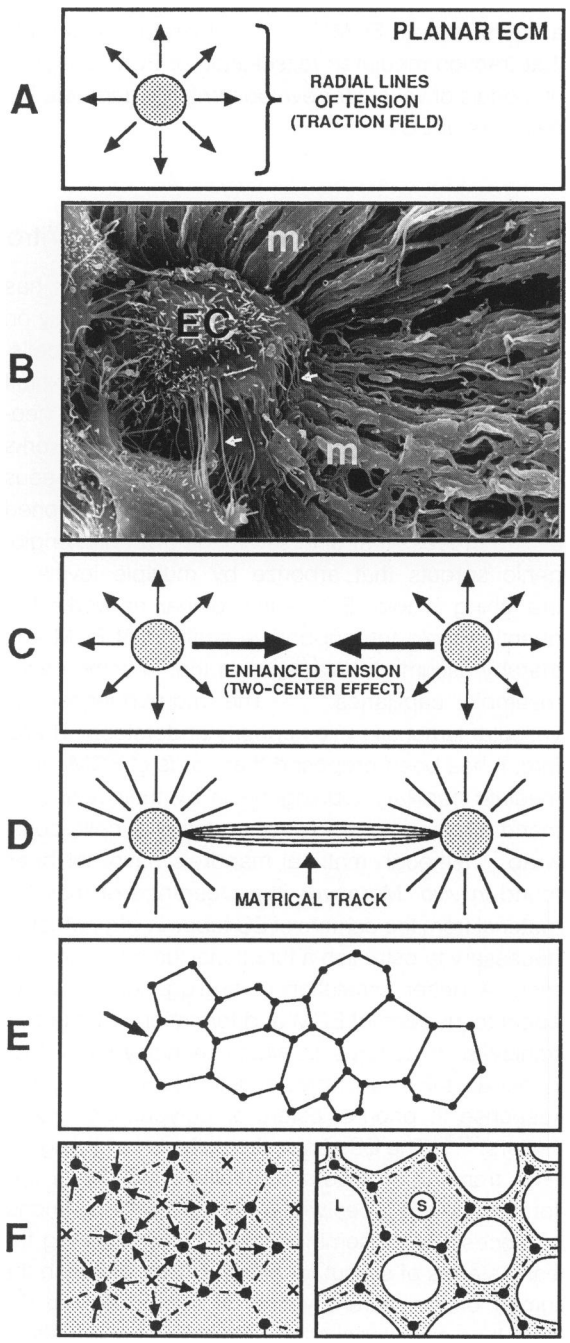

Figure 3. *Cellular traction generates patterns of order in planar ECM* in vitro. A: *Viewed from above, a cellular traction center* (shaded circle) *in contact with malleable, planar ECM* (rectangle) *generates radial lines of tension* (arrows) *in the ECM that constitute a traction field.* B: *An EC cultured on a layer of Matrigel is seen by scanning electron microscopy* (×2400 *at 60° of tilt*). *Ribbons of Matrigel* (*m*) *that comprise a traction field radiate from beneath the EC. Long microvilli* (arrows) *extend from the edge of the EC and contact the ECM.* C: *Tension in ECM between adjacent traction centers is enhanced* (large arrows) *as a consequence of the two-center effect.* D: *Fibers of ECM* (black lines) *align along the direction of principal stress. Fibers influenced by the two-center effect align to form a matrical track that connects the traction centers.* E: *Traction centers* (small black dots) *arranged in a field on planar ECM become connected by matrical tracks* (*eg,* arrow) *that form a network.* F, left panel: *Where planar ECM* (*shaded*) *is highly malleable, centripetal movement* (arrows) *of ECM to traction centers* (black dots) *results in clearance of ECM from central areas* (*x*) *that are bordered by two-center effects* (dotted lines). F, right panel: *Clearance process diagrammed in left panel is manifested as perforations* (*white areas*) *in the sheet of ECM* (*shaded*). *Perforations are small* (*S*) *initially but enlarge with time* (*L*). *ECM aligned by two-center effects* (dotted lines) *between traction centers* (black dots) *is resistant to cell-generated stresses.* A *and* C–F *are reprinted from Vernon et al.*[16] *Copyright © 1995 by the Society for In Vitro Biology. Reproduced with permission of the copyright owner.*

Traction and Morphogenesis in Vivo

Despite the limitations of planar culture systems, it is premature to dismiss traction-mediated phenomena as artifacts of culture. Although traction and progressive motility are mediated *via* the cytoskeleton, it is unlikely that traction is a by-product of cellular migration. Cells that move rapidly *in vitro* (eg, macrophages and polymorphonuclear leukocytes) distort malleable substrates minimally *in vitro*.[24,26] Moreover, slower moving cells such as fibroblasts exert forces *via* traction that are 100- to 1000-fold greater than is necessary to propel the cells at normal speeds *in vitro*.[24] It is thought that such high levels of traction allow fibroblasts to compress and align ECM during the repair of wounds[37] and during the development of dense connective tissues such as tendons, ligaments, and organ capsules.[25]

The traction of ECs *in vitro* is similar in magnitude to that of dermal fibroblasts[38]; therefore, it is plausible that ECs restructure ECM *via* traction for purposes of morphogenesis *in vivo*. Levels of traction applied by various human melanoma cell lines to type I collagen *in vitro* were correlated positively with their invasion of ECM and metastasis *in vivo*.[39] Correspondingly, we observe that traction-mediated contraction of type I collagen gels by ECs *in vitro* is stimulated by basic fibroblast growth factor,[38] which enhances angiogenic sprouting *in vivo*[40] and promotes invasion of collagen by ECs *in vitro*.[41] The function of traction in matrical invasion during angiogenesis is unclear; traction might contribute directly to the propulsion of ECs through ECM and/or facilitate the reorganization or clearance of ECM immediately ahead of invading ECs. Application of high levels of traction force by sprouting ECs might align ECM over greater distances and thereby form pathways for cellular migration, a process associated with the movement of endocardial cells into the endocardial cushions of the developing chicken heart.[42] Indeed, the existence of pathways of ECM is consistent with the follow-the-leader behavior exhibited by ECs that sprout *in vivo*[43] and *in vitro*.[44] Matrical pathways could also facilitate the development of anastomoses between vascular sprouts (a common occurrence *in vivo*); ECs at the tips of adjacent sprouts would align ECM between them by a traction-mediated two-center effect, approach one another *via* the matrical pathway, and fuse to establish a common lumen (Figure 4).

Beyond the role of cellular traction in the reorganization of vicinal ECM *in vivo*, the potential for traction/tessellation systems to generate large-scale multicellular patterns in tissues with planar architectures warrants further consideration. For example, tessellated fields of mechanical strain that are generated in dermal ECM by the traction of primordial dermal papillae have been implicated in the arrangement of feather germs and the associated network of connective musculature in avian skin.[45,46] With regard to vascular development, a variety of microvascular systems exhibit planar characteristics *in vivo* that render them amenable to morphogenesis *via* traction, for example, the networks of microvessels within the early avian embryo and its extraembryonic membranes, capillary plexuses of acinar exocrine glands and pulmonary alveoli, and microvasculature of the eye. Indeed, the development of the chicken and quail para-aortic and vitelline vascular plexuses from a field of dispersed angioblasts closely resembles the development of planar networks of ECs *in vitro* (Figure 5).[16,47] The recently proposed concept of vascular growth in the absence of angiogenic sprouting, termed intussusception, involves the expansion of planar capillary beds (eg, in the growing rat lung) *via* the perforation of dilated or sheetlike areas of vasculature and their subsequent conversion into networks of tubes.[48,49] Observations of planar models *in vitro* indicate that intussusception can be mediated *via* tension; when the mechanical strength of a sheet of cells and/or ECM is exceeded by applied tension, reorganization of the sheet into a network of cords is initiated when punctate mechanical failures arise and enlarge as circular perforations (Figure 3F).[16,21,25] Intussusceptive growth *in vivo* might also occur in capillary networks with a three-dimensional arrangement,[49] a circumstance that does not rule out a role for tension, which is readily applied in three dimensions. Sources of tension that could mediate vascular intussusception *in vivo* include (1) the traction of ECs within vasculature that is prevented from retracting because of its anchorage to surrounding tissues, (2) penetration of a vascular network by ECM, or (3) the general expansion of tissues (eg, *via* growth) to which the vasculature is peripherally attached. Intussusception is a dynamic process that will be difficult to identify and to study *in vivo*; therefore, it will be necessary to use traction/tessellation models *in vitro* to dissect the relevant mechanical and biochemical mechanisms.

Control of the Pull: A Relevance to Vascular Morphogenesis?

It is of critical importance that mechanisms of development be regulated. For morphogenetic processes that involve the restructuring of ECM, the equilibrium between cellular traction and the opposing viscoelastic resistance of ECM to deformation will determine the magnitude of matrical reorganization. Gene products that would shift this equilibrium include those that affect (1) the traction-generating elements of the cytoskeleton, (2) the mechanical properties of the ECM, or (3) the coupling, *via* transmembrane cell surface molecules, between the force-generating elements of the cytoskeleton and the ECM.

Alteration of the mechanical properties of ECM has significant effects on its response to cellular traction *in vitro*. For example, decreasing the malleability of Matrigel by reducing the depth of the layer over a rigid support (eg, glass) inhibits the alignment of ECM by cells and prevents the development of cellular networks.[21] Similarly, the cell-mediated reorganization of gelled fibrillar type I collagen *in vitro* is

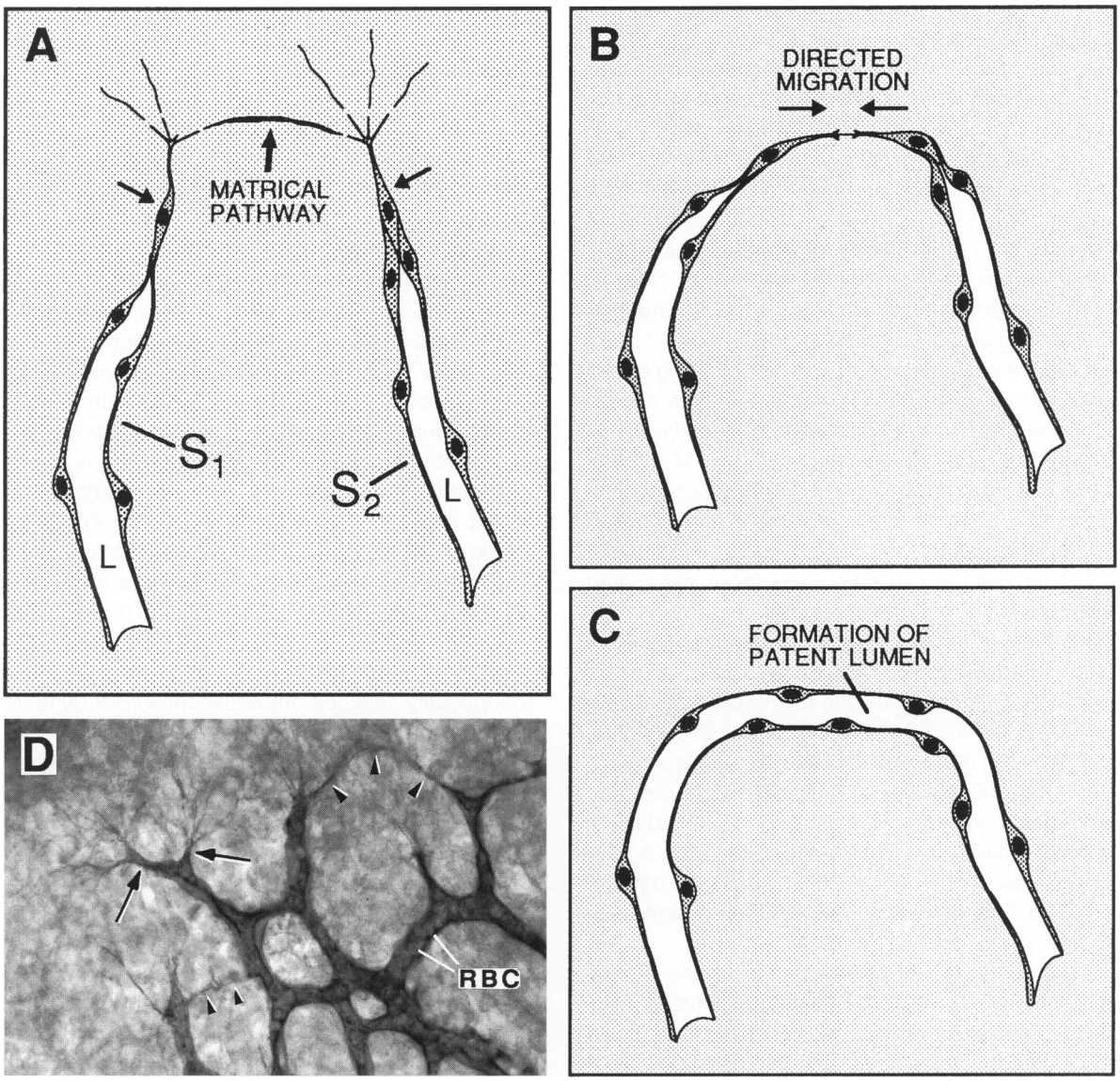

Figure 4. *Hypothetical role of matrical pathways in the anastomosis of angiogenic sprouts* in vivo *is shown in diagrams* **A–C** *(clockwise from left).* **A:** *Migratory ECs* (**small arrows**) *at tips of adjacent sprouts (S_1, S_2) create a connecting pathway of aligned ECM (matrical pathway) as a consequence of a traction-mediated two-center effect. Lumens (L) of sprouts are indicated.* **B:** *Migratory ECs approach one another* (**arrows**) via *the matrical pathway.* **C:** *The vascular loop is completed as ECs meet, adhere, and interact to form a patent lumen.* **D:** *Advancing front of the planar capillary network within the nerve fiber layer of a fetal Macaca monkey retina (day 105 of gestation). Capillary sprouts with patent lumens that contain red blood cells (RBC) are labeled darkly with an immunoperoxidase reaction for the cell adhesion molecule CD31, a marker for ECs. ECs at tips of sprouts* (**arrows**) *extend filamentous, cytoplasmic processes. Matrical pathways might facilitate the formation of cytoplasmic bridges* (**arrowheads**) *that initiate anastomosis.* **D** *(×250) is courtesy of R. F. Gariano, Department of Ophthalmology, University of Washington.*

suppressed as the concentration of collagen in the gel is increased.[21,38] Potentially, cells can regulate the mechanical properties of vicinal ECM by the secretion of molecules with specific physical characteristics. We observe that the spontaneous development of matrical networks by bovine aortic ECs *in vitro* requires the secretion of type I collagen by these cells; cultures that lack this collagen do not generate networks of ECM.[16] Fibrils of type I collagen are well suited for reorganization by traction because elastic interactions between the fibrils allow the transmission of stress over significant distances. Conversely, secretion of fibrillar collagens by cells is likely to increase the tensile strength of the surrounding ECM as a consequence of physical entanglement of the fibers and of the presence of covalent cross-links between fibers. The stiffening properties of fibrillar collagens might be important for vascular growth and stability; inhibitors of collagen deposition and cross-linking induce regression of capillary net-

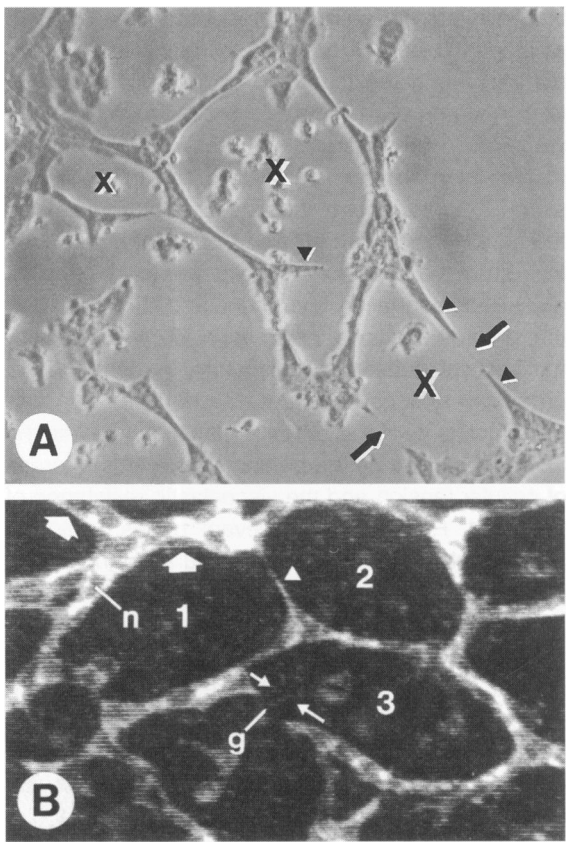

Figure 5. *Behavior of ECs on matrical scaffolds* in vitro *resembles the cytoarchitecture of vascular development* in vivo. **A:** *Bovine capillary ECs are assuming a tessellated pattern on thin strands of fibrillar type I collagen. Geometric centers (X) of three polygonal tesserae are indicated.* **Arrows** *indicate areas where the strands of collagen are most clearly visible in this phase-contrast image. ECs migrating on the ECM exhibit tapered morphologies* (**arrowheads**). **B:** *Arrangement of ECs in the developing para-aortic vasculature of a quail embryo (six-somite stage) is revealed by immunofluorescence and laser confocal microscopy. ECs are labeled with an antibody to QH1, a marker for primordial ECs. Geometric centers of three vascular polygons are indicated (1, 2, and 3). Polygon 3 has an acellular gap (g) with the potential for closure by migration* (**small arrows**) *of two opposed ECs. An initial stage of network closure is represented by the thin, cellular extension* (**arrowhead**) *that separates polygon 1 from polygon 2. Thicker, multicellular cords* (**large arrows**) *comprise areas of greater maturity. Nuclei (n) of endothelial cells appear as dark ovals.* **A** (×100); **B** (×145) *is reprinted from Vernon et al.[10] Copyright © 1995 by the Society for In Vitro Biology. Reproduced with permisston of the copyright owner.*

works in the growing chick chorioallantoic membrane, presumably because a mechanically stable substrate to which the growing capillaries can anchor is not formed.[50]

Evidence indicates that the secretion of proteases by ECs is an important element of the angiogenic process. For example, inhibition of endogenous metalloproteinases blocks angiogenesis within the chick chorioallantoic membrane,[51] suppresses the invasion of type I collagen gels by tubular sprouts of human ECs *in vitro*,[52] decreases the contraction of type I collagen gels by bovine ECs *in vitro*,[38] and inhibits the organization of ECs into networks on Matrigel *in vitro*.[22] It has been proposed that proteolysis of ECM facilitates angiogenesis through the release of bound molecular factors that promote vascular growth; however, it is likely that the primary effect is the reduction of the mechanical resistance of ECM to penetration and reorganization by migratory ECs.[53] The importance of proteolysis to angiogenesis is underscored by the observation that two potent stimulators of angiogenesis *in vitro* and *in vivo*, basic fibroblast growth factor and vascular endothelial growth factor, enhance the degradation of ECM by ECs through their increased synthesis of plasminogen activator, collagenase, and other proteases.[5] It appears, however, that the range of matrical proteolysis that is angiogenic has an upper limit; for example, the inhibition of tubulogenesis *in vitro* by murine ECs that express the polyoma middle T oncogene is believed to be caused by excessive mechanical disruption of their supportive fibrin matrix by endogenous proteases.[54] Maintenance of a range of matrical malleability that is optimal for invasion, growth, and morphogenesis during neovascularization is likely to reflect a balance between the degradation of ECM (a function of the interactions between proteases and their cognate inhibitors) and synthesis of new ECM. This balance might be achieved, in part, *via* opposed effects of angiogenic factors; in contrast to basic fibroblast growth factor and vascular endothelial growth factor, the angiogenic transforming growth factor-β enhances the integrity of ECM through its inhibition of proteolysis and stimulation of ECM synthesis. Transforming growth factor-β inhibits synthesis of plasminogen activator and stimulates synthesis of plasminogen activator inhibitor 1 by capillary ECs.[55] Moreover, transforming growth factor-β increases the secretion of tissue inhibitor of metalloproteinases by fibroblasts[56] and stimulates fibroblasts to synthesize structural components of ECM such as collagen and fibronectin.[57–59]

For traction-mediated interactions between ECs and ECM to occur, forces developed by the cytoskeleton must be transmitted across the plasma membrane to the ECM. Mediators of this process include the integrins, which are transmembrane receptors that physically link actin-associated proteins, such as talin, vinculin, α-actinin, and paxillin, to ECM.[60–62] Integrins are a family of molecules comprised of at least 20 heterodimers formed from noncovalently associated α- and β-subunits; it is the combination of a particular α- and β-subunit that determines, to a large extent, receptor specificity. Integrins that are important mediators of cellular attachment to type I collagen include $\alpha_1\beta_1$, $\alpha_2\beta_1$, and

$\alpha_3\beta_1$. Integrin $\alpha_2\beta_1$ is associated with traction-mediated reorganization of type I collagen by human fibroblasts[63] and by metastatic human melanoma cell lines,[39] as shown by inhibition of collagen gel contraction with specific antibodies *in vitro*. Accordingly, the synthesis of integrin $\alpha_2\beta_1$ (but not of integrins $\alpha_1\beta_1$ or $\alpha_3\beta_1$) is selectively increased among fibroblasts grown on collagen gels.[39] Moreover, the stimulation of β_1 integrin-mediated collagen gel contraction among fibroblasts *in vitro* by angiogenic platelet-derived growth factor[64] is accomplished by a selective increase in the synthesis of the α_2 subunit.[65] Integrin $\alpha_2\beta_1$ is present on capillaries *in vivo*[66] and on isolated microvascular ECs *in vitro*.[67] The reorganization, by EC monolayers, of solubilized fibrils of type I collagen into a planar, cell-bearing network *in vitro* is inhibited by antibodies against integrin $\alpha_2\beta_1$;[68] therefore, it is likely that this integrin plays an important role in the application of traction to type I collagen by ECs *in vitro*. Although $\alpha_2\beta_1$ is a major factor in the attachment of umbilical vein ECs to laminin *in vitro*,[69] it plays little or no role in the traction-mediated organization of umbilical vein ECs into networks on Matrigel *in vitro*,[23] a process dependent on the laminin-binding integrin $\alpha_6\beta_1$.[23,70] Expression of a number of integrins of the β_1 subfamily ($\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_5\beta_1$, and $\alpha_6\beta_1$) on capillary ECs *in vitro* is stimulated by basic fibroblast growth factor[67]; however, the functions of β_1 integrins in vascular morphogenesis *in vivo* remain unclear. A monoclonal antibody (CSAT) against avian β_1 integrins induces abnormalities of aortic vasculogenesis in the quail embryo that include inhibition of luminal development[47]; however, CSAT does not inhibit angiogenesis in the chick chorioallantoic membrane despite the presence of β_1 integrins in nascent vessels.[71] In contrast, angiogenesis in the chick chorioallantoic membrane is inhibited by antibodies against $\alpha_v\beta_3$,[71] an integrin that interacts with a variety of ECM components that include fibrinogen, von Willebrand factor, vitronectin, and denatured type I collagen.[72,73] Integrin $\alpha_v\beta_3$ is expressed preferentially by angiogenic vasculature[71,74] and, therefore, might function during the invasion of ECM by ECs, a hypothesis supported by the finding that human melanoma cells with an enhanced potential for metastasis exhibit elevated levels of $\alpha_v\beta_3$ *in vitro*[75] and *in vivo*.[76]

Summary: Between Molecules and Morphology

In response to an angiogenic stimulus, ECs initiate programs of gene expression that result in the quantitative alteration of gene products within nuclear, cytoplasmic, cell surface, and extracellular compartments. During the formation of new microvasculature, patterns of molecular expression among individual ECs must direct the creation of complex, multicellular morphologies in two and three dimensions. Studies *in vitro* indicate that cell-generated forces of tension can organize ECM into structures that direct the behavior of single cells (*via* influences on cellular elongation, alignment, and migration) and that provide positional information for the creation of multicellular patterns. Significantly, the formation of organized matrical structures is controlled by gene products (of ECs or other cell types that populate the ECM) that influence the balance between the forces of cellular tension and the viscoelastic resistance of the ECM. Regulation of relevant genes could be accomplished by soluble molecular signals (eg, growth factors) and/or solid-state signals arising from specific arrangements of cytoskeletal structure that, in turn, are a function of the equilibrium between cellular tension and matrical resistance.[77] Within cells, information for the construction of complex organelles is encoded in the shapes of the constituent molecules. Similarly, the creation of complex vascular architecture must be mediated by molecular shapes, a fact that is readily apparent in simple receptor-ligand interactions such as the binding of growth factors to ECs or the attachment of ECs to one another. However, between molecules and morphology also exists a set of multilayered, interactive, multimolecular systems that establish vascular form at unicellular and multicellular levels. Characterization of these systems is an elusive target that resides at the frontier of vascular biology; the identification of models *in vitro* that accurately reproduce macroscale events of vascular morphogenesis should advance considerably our understanding of vascular development and lead to an elucidation of its regulation *in vivo*.

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