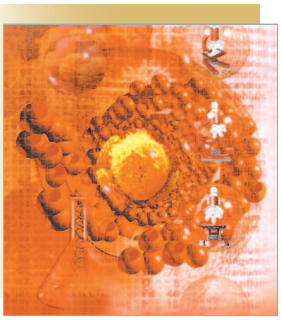

Multiscale Models of Angiogenesis

Integration of Molecular Mechanisms with Cell- and Organ-Level Models

BY AMINA A. QUTUB, FEILIM MAC GABHANN, EMMANOUIL D. KARAGIANNIS, PRAKASH VEMPATI, AND ALEKSANDER S. POPEL

Vascular disease, cancer, stroke, neurodegeneration, diabetes, inflammation, asthma, obesity, arthritis—the list of conditions that involve angiogenesis reads like main chapters in a book on pathology. Angiogenesis, the growth of capillaries from preexisting vessels, also occurs in normal physiology, in response to exercise or in the process of wound healing. Why and when is angiogenesis prevalent? What controls the process? How can we intelligently control it? These are the key questions driving researchers in fields as diverse as cell biology, oncology, cardiology, neurology, biomathematics, systems biology, and biomedical engineering. As bioengineers, we approach angiogenesis as a complex, interconnected system of events occurring in sequence and in parallel, on multiple levels, triggered by a main stimulus, e.g., hypoxia.

In response to hypoxia, the transcription factor hypoxia-inducible factor 1 (HIF1) activates hundreds of genes [1], [2], including vascular endothelial growth factor (VEGF). VEGF proteins stimulate chemotaxis and proliferation in endothelial cells (ECs) during capillary sprouting. There are seven known isoforms of VEGF, each with a different effect on cell behavior, and ultimately, on vascular pattern formation [3]–[5].

Although VEGF is a main angiogenic growth factor, numerous other proangiogenic factors exist, including fibroblast growth factor, angiopoietin, tumor necrosis factor, and transforming growth factor. The balance of proangiogenic factors with antiangiogenic factors, e.g., endostatin, thrombospondin-1, and angiostatin, controls the extent of microvascular growth. At the onset of angiogenesis and throughout the process, cell activation, cell migration, and cell proliferation are a function of local growth factor concentrations and gradients (Figure 1). Furthermore, angiogenic sprouting also involves cell–matrix interactions by a complex synergy of chemical and mechanical cues. Matrix metalloproteinases (MMPs) are the key molecules involved in the process of an activated cell proteolyzing its surrounding extracellular matrix (ECM), forming a moving sprout tip, and releasing ECM-bound factors as it migrates. Once a capillary sprout develops, its fate is determined by the surrounding vascular network and tissue: it can anastomose and attach to adjacent vessels; it can retract; it can split or it can branch. As sprouts form and connect, a new capillary network arises, eventually capable of carrying blood and bringing oxygen to hypoxic regions.

To put the complexity of this process into perspective, at the molecular level, various degrees and durations of hypoxia yield different activity of HIF degradation enzymes, HIF synthesis and reactive oxygen species (ROS), and hence oxygen sensitivity; there are three known isoforms of HIF, three main isoforms of HIF prolyl hydroxylases (PHDs), seven isoforms of one VEGF gene, five VEGF genes, five VEGF cell-surface receptors [5], three isoforms of MMP2, one of the 26 MMPs [6], and hundreds of peptides endogenous to the local matrix capable of altering cell migration or proliferation. At the single and multiple cell level, other ligand–receptor combinations such as notch and delta-like ligand 4 (Dll4) change tip cell density and capillary branching properties [7]. Furthermore, the position of a cell on a capillary sprout determines what growth factor concentrations it sees, its activation and protein expression, and its locomotive properties [8], [9]. In addition, while ECs are the cellular focus for this review, pericytes, precursor cells, and stromal cells, among others, play important roles in cell signaling, growth factor production, EC movement, vessel stability and capillary permeability [10]. Finally, tissue composition and heterogeneity ultimately determine network structure, and paracrine signaling from different organs and organ systems, blood flow, inflammatory response, and lymphatics can alter angiogenesis [10]. Not only is multiscale modeling a useful tool in determining how these factors interact, deciphering new experimental findings in angiogenesis and proposing new experiments, one could argue it is an essential tool to truly understand a process of such biological complexity.

Modeling Approaches

Before describing modeling approaches, we first define what we mean by multiscale modeling. In our context, it is computational modeling across levels of biological organization, with respect to space or time. We revolve our discussion around four main biological spatial levels: molecule (subcellular), cell, tissue, and organ system. For further explanation of multiscale, recent reviews have been devoted to defining multiscale in biological systems, and offered detailed critiques of linking between scales [11], [12]. Here we focus strictly on multiscale models of angiogenesis and their integration.

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The basis of this review is molecular models and how multiscale modeling emerges from their development. However, even before VEGF or HIF1 or the MMPs were isolated and characterized, computational modeling of microvascular growth had begun. Mathematical representations of angiogenesis date to the 1970s and earlier, and some of the first models used differential equations to represent a generic growth factor as a chemotactic stimulus, produced and released by a tumor mass, and inducing growth of vessels into the tumor [13]–[15]. Models have since included detailed equation-based network models of tumor-induced angiogenesis [16], a model of capillary growth in a corneal pocket assay [17], molecular level interactions of VEGF and its receptors coupled to vessel oxygenation [18], a cell-level rule-based model of network growth in mesenteric tissue [19], Potts models of angiogenic and vasculogenic growth [20], [21], network formation stemming from capillary movement through a matrix composed of aligned collagen fibers [22], VEGF-driven angiogenic growth applied to a vascular engineering construct environment [23], among many others [24]–[26]. Each model brings a new perspective on the biological phenomenon behind neovascularization, and together, they give insight on the multiple conditions affecting angiogenesis and the multiple conditions affected by angiogenesis. Here, we describe angiogenic processes modeled at different biological scales, introduce diverse types of computational models, report on progress toward integrating multiscale representations, and summarize examples of multiscale model applications from work in our laboratory and from other investigators in the field. In this brief review, we do not attempt to cover all existing models of angiogenesis nor provide a comprehensive analysis of multiscale modeling approaches. Throughout the text and in the concluding summary, we list several additional resources and reviews that delve deeper into subjects covered in this article.

Angiogenesis

Multiscale Modeling

Table 1 highlights the models of angiogenesis and their properties across the molecular, cellular, tissue, and organ system levels. Reaction and reaction-diffusion-based differential equation approaches using the continuum approximation so far have the dominated models of angiogenesis; they are the first method to have been used at all four biological scales. In areas where differential equations have limited utility, other methodologies have been introduced. Discrete models have the benefit of being able to represent biological components as single, complete entities with guiding properties and rules that can be independent of adjacent compartments—this has been primarily applied at the cellular level, where cells are the discrete objects [19], [20], [27]–[29] and growth factor gradients may be discretized from continuous equations. The utility of hybrid approaches is being explored with emerging multiscale models that can couple previously developed detailed, differential equations with discrete representations of growth factors, cells, ECMs or tissues. A benefit of the hybrid approach is that it is intrinsically designed with modularity, whereas continuous models may need significant internal code changes, e.g., to introduce a new molecular compound involved in binding reactions, and for example, discrete models may introduce error in their assumption that cells are in a single discrete state at every time step. Besides model integrative properties, the biological levels of interest, the hypotheses being tested, the availability and type of experimental data, and spatial resolution determine the optimal methodology. Such diverse approaches as fractals and wave equations have characterized patterns in capillary formation and offered phenomenological hypotheses about the system that other methodologies would be unable to capture. Logic-based models have also emerged in angiogenesis and tumor growth models as a means to represent biological processes as rules. Rules can be of any form expressed logically or mathematically—equations, Boolean rules, and probabilities are examples employed so far.

The degree of complexity introduced in angiogenesis models can be designed to parallel or extend beyond the biological resolution attainable by experiments. High model resolution comes at the cost of requiring exhaustive literature searches or experimental comparisons and parameter analysis. Like other models, all multiscale modeling approaches risk under- or overfitting of

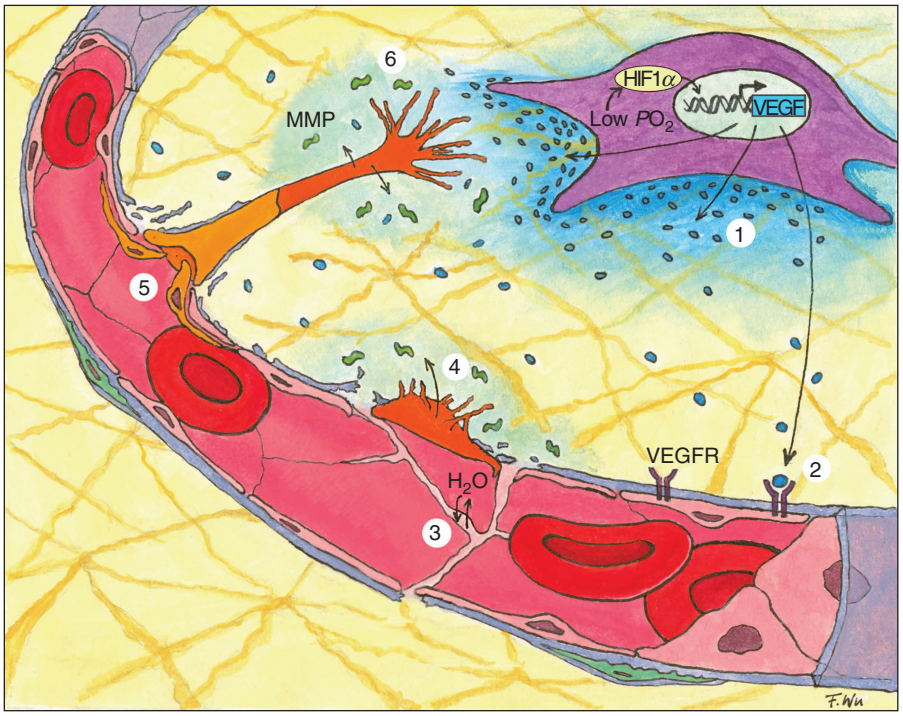

Fig. 1. Schematic of processes involved in angiogenesis. 1) Hypoxia-inducible factor 1 (HIF1) is upregulated in a hypoxic cell. HIF1 activates the transcription of vascular endothelial growth factor (VEGF), which is then secreted by the cell. 2) VEGF-VEGFR binding on the capillary surface. 3) Vessel permeability changes. 4) An activated EC (the tip cell) starts to break down the basement membrane. 5) Stalk cells proliferate behind the tip cell. 6) The leading edge of the moving sprout releases MMPs which proteolyze the surrounding extracellular matrix, allowing the cell to migrate.

Table 1. Computational models representing angiogenesis on multiple biological scales.

Model Methodology		Properties Explored by Models (Categorized by Biological Level)			
		Molecular	**Cellular**	**Tissue**	**Organ System**
Discrete	Monte Carlo (126)	• VEGF molecular interactions • VEGF–VEGFR interactions			
	2-D agent-based (19), (127)	• VEGF • FGF • PDGF	• EC, SMC, pericytes, and inflammatory cells • Cell migration • Cell proliferation • Chemotaxis • Cell cycle	• Branching • Capillary network formation	
	Potts model (20), (21)	• VEGF • VEGF diffusion	• ECM interaction	• Branching • Capillary formation	
	2-D random walk-based models (23), (29), (128)	• FGF • VEGF • VEGF diffusion, degradation, and production	• Cell migration and persistence • Chemotaxis • Cell density	• Branching • Capillary network formation in tissue engineering construct	
Hybrid	3-D agent-based	• VEGF • VEGF gradient • Dll4 presence	• EC activation • EC migration and persistence • EC proliferation • ECM interactions • Chemotaxis	• Branching • Capillary formation • $Dll^{-/+}$, $Dll^{-/+}$	
	2-D discrete cell movement, continuous cell environment interactions (28), (129), (130)	• Generic TAF • Angiostatin	• EC migration • Pericytes, macrophages • Cell–matrix interactions • Cell-surface receptor binding • Matrix degradation • Chemotaxis	• Branching • Capillary network formation	
Continuous	Reaction-diffusion (15), (18), (31), (40), (47), (61), (68), (70), (75), (81), (107), (108), (114), (131)–(134)	• MMP interactions • HIF1α degradation • VEGF molecular interactions • VEGF–VEGFR, VEGF–neuropilin interactions • Generic TAF	• ECM interactions • MMP-driven release of VEGF • Matrix heterogeneity • HIF1α autocrine synthesis	• VEGF • VEGF–VEGFR interactions • Blood flow • O_2 transport • Vessel adaptation	• VEGF • VEGF organ system distribution
	Wave equations (24), (133)	• Generic TAF	• ECM interactions • EC proliferation • EC migration • Cell-surface receptor binding	• Capillary network formation	
	Fractals (125), (135)			• Capillary network formation	• Tumor tissue versus adjacent normal brain vascular network formation

parameters. This effect is most relevant to multiscale, integrative modeling, where error could be propagated between models and across scales. That said, benefits of model integration outweigh costs. Existing models approach different pieces of the angiogenic process (Figure 1): blood flow to oxygen transport, hypoxic sensing to growth factor secretion, growth factor signaling to cell migration and proliferation, and neovascular formation to new capillary networks. Figure 2 shows current modules developed in our laboratory, and the aim and breadth of possible integration. Later, we introduce these angiogenesis models, describe their applications at different biological levels, and discuss routes for combining their predictive abilities. Throughout this article, labeled schematics are used to illustrate the design of the models, while graphs highlight results, and other figures show computational predictions of vessel properties. So far, most angiogenesis models are neither highly integrative nor used outside a single laboratory. It is the aim of this article to foster integration of many existing and future models with the work described here.

Blood Flow and Oxygen Transport

Most commonly, angiogenesis starts where there is insufficient blood flow to meet a tissue's metabolic demand for oxygen. Models of microvascular blood flow and oxygen transport have been described in numerous publications from different laboratories. A recent review on the subject describes the state of the art in the field [30]. Briefly, a three-dimensional (3-D) geometrical model of a microvascular network in a tissue volume is constructed. Given appropriate boundary conditions (pressure or flow),

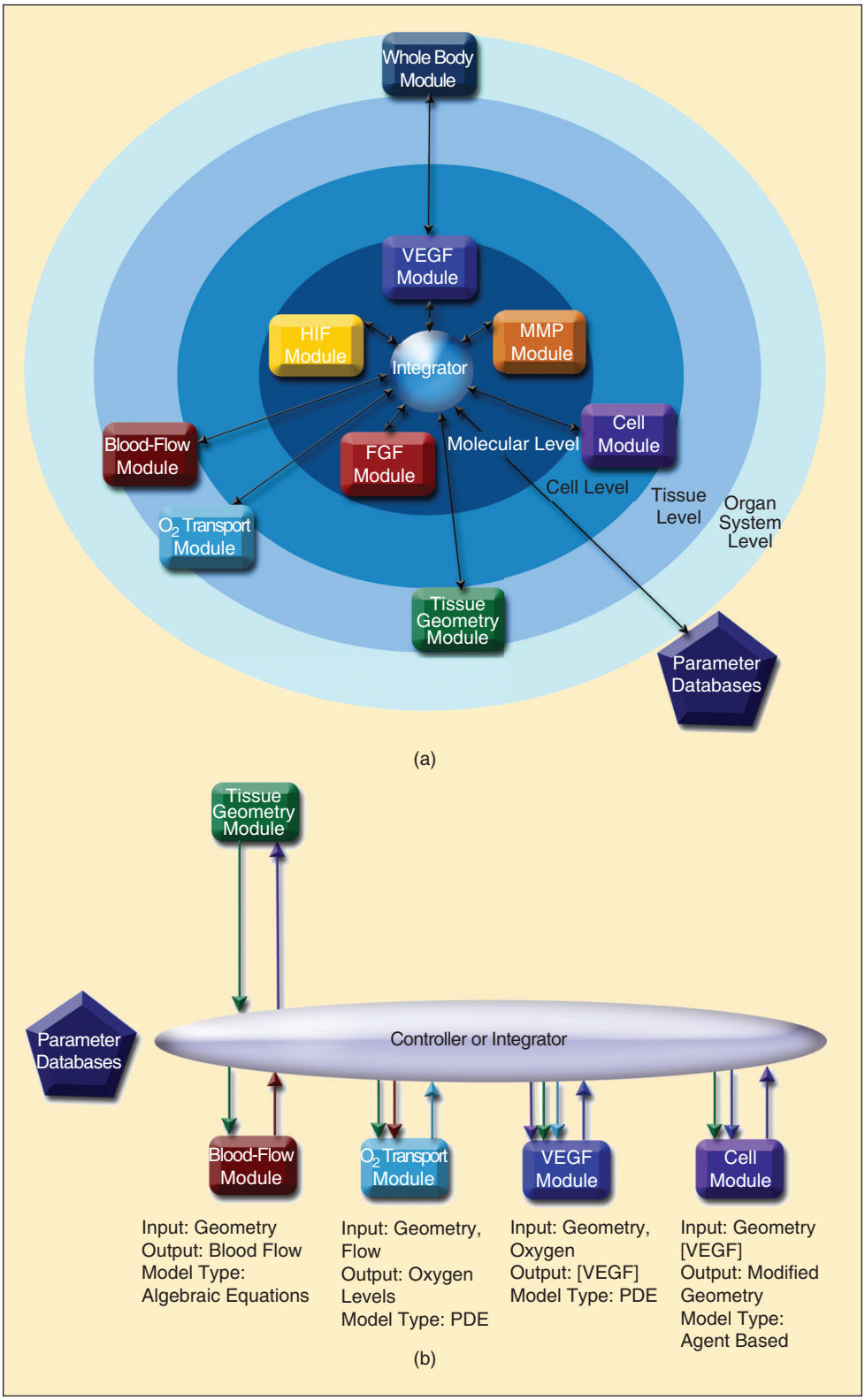

Fig. 2. Schematics illustrating the design and implementation of multiscale models of angiogenesis. (a) Proposed integration of angiogenesis modules developed in our laboratory. Current modules are shown in rectangles, placed spatially on the ring representing the main biological level the models address. An integrator is being developed to regulate scheduling and data passing between any two or more models. A database stores parameters such as kinetic rates, organized by conditions and microenvironment (e.g., in vivo, ischemic). (b) A flow diagram showing the integration of four models currently being developed. The controller (a type of integrator) passes all data, runs programs, and controls scheduling.

blood flow and hematocrit distribution can be calculated throughout the network by solving a set of nonlinear algebraic equations for pressure at the network nodes (bifurcations) and blood flow rate and hematocrit in the vascular segments. An example of such computer simulations is shown in Figure 3 (modified from [31]). Having simulated blood flow in the network, convection-diffusion-reaction partial differential equations governing oxygen transport are solved numerically, resulting in 3-D distribution of oxygen in the microvascular network and surrounding tissue [32]. An example is given in Figure 4(b) for the skeletal muscle under normal and low-oxygen exercising conditions (left panels). The calculated detailed distribution of oxygen concentration provides a basis for modeling cellular response to oxygen via HIF.

Oxygen to HIF1 Signaling

Transient Hypoxia: Molecular Level

Cellular response to oxygen hinges in large part on the transcription factor HIF. HIF1, the first characterized member of the HIF family, is a heterodimer composed of subunits HIF1α and HIF1β. The β subunit is constitutively expressed in cells. Expression of the α subunit may be induced by a number of pathways, and its degradation is highly sensitive to O_2 levels. Called a master switch for hypoxic gene expression [1], [33], intracellular HIF1α is experimentally undetectable under normoxic conditions; during hypoxia, it rapidly accumulates in the cell nucleus and triggers gene expression. Hundreds of genes associated with angiogenesis in cancer, exercise and ischemia, energy metabolism, nutrient transport, cell cycle, and cell migration are activated by HIF1 [1], [34].

Therapeutic Use of Molecular Cofactors in the HIF1 Regulatory Pathway

Considering HIF1's transcriptional activity, therapeutic regulation of the HIF1 pathway presents an attractive way to control angiogenesis [35]–[39]. Cofactors in HIF1 degradation are prime molecular level targets; these include PHDs, iron, ascorbate, hydrogen peroxide, 2-oxoglutarate (2OG), succinate (SC), and von Hippel-Lindau protein [Figure 5(a)]. Computational modeling was used to test two possible molecular therapies in conditions of cellular hypoxia—therapies supplementing with ascorbate alone, and the combination therapy of supplementing with iron and ascorbate [Figure 5(b)] [40]. Both decreased HIF1α expression during hypoxia, and where iron was in limited supply, the model showed that ascorbate had a significant effect in modulating oxygen response and HIF1α expression. The utility of ascorbate supplementation, through its HIF1 hydroxylation role, has now been validated in vivo and has been shown to inhibit tumor growth [41].

Chronic Hypoxia: Molecular Level

Hypoxia occurs in cancer, prolonged exercise, and long-term ischemia with durations of several hours or more. Under these conditions, the threshold of hypoxic response changes. Mammalian cells exposed to chronic hypoxia (5% oxygen) and then exposed to a lower level of oxygen (0.5%) are capable of showing a response consistent with acute hypoxia but attenuated [42]. Additionally, hypoxic preconditioning contributes to a limited hypoxic response in reoxygenated cells [43] and shows protective effects in mammals exposed to ischemia [44]. Hydroxylation enzyme synthesis and its effect on degradation of HIF1α contribute to this set point adjustment [42], [43], [45], [46]. We computationally tested the hypothesis that three feedback loops (HIF1α synthesis, PHD synthesis, and SC production inhibition) work in combination to tightly regulate the effects of chronic hypoxia via control of HIF1α degradation [47]. Results demonstrate that PHD, SC, and HIF1α feedback determine intracellular HIF1α levels over the course of hours to days. The model provides quantitative insight critical for characterizing molecular mechanisms underlying a cell's response to long-term hypoxia.

Hypoxia, HIF1, and ROS

There are several hypotheses as to how ROS interact with the HIF1 pathway and alter HIF1α expression (recent related reviews include [48], [49]). One possibility is that hydrogen peroxide oxidizes ferrous iron (Fe^{2+}) to its ferric form (Fe^{3+}), preventing the necessary binding of ferrous iron to the HIF1α hydroxylation enzymes PHDs [50] [Figure 5(a)]. Another

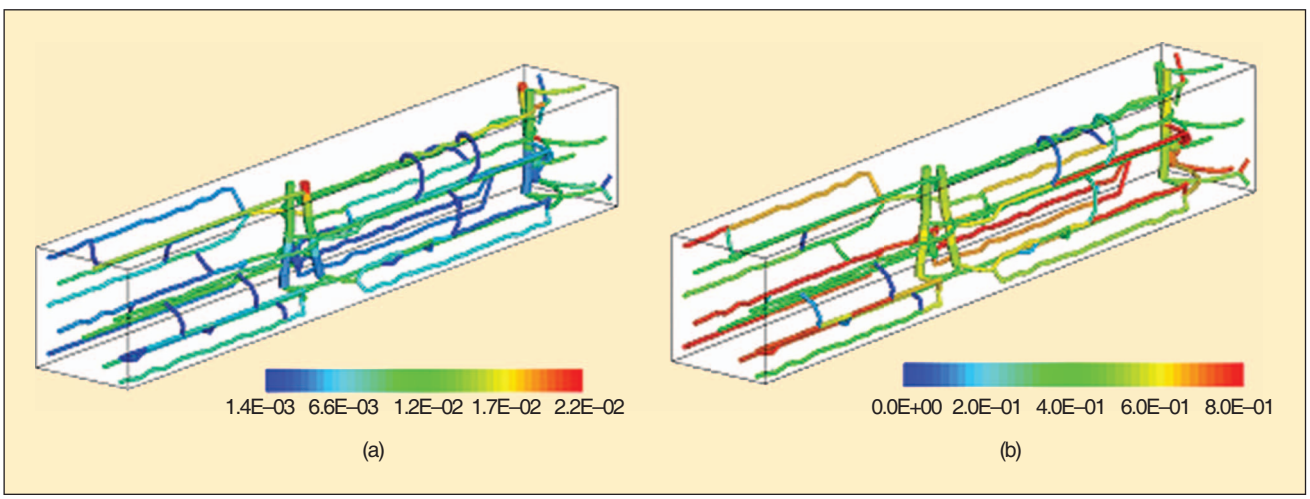

Fig. 3. (a) Velocity and (b) hematocrit distributions for a representative 100 μm by 100 μm by 800 μm capillary network in rat skeletal muscle. The figure is adapted from (31) with permission from Elsevier, where the equation-based models representing blood flow and oxygen transport are discussed. Average vessel geometries were approximated from skeletal muscle experiments, and a stochastic algorithm based on geometric constraints was then used to generate the set of capillaries shown, as described in (31).

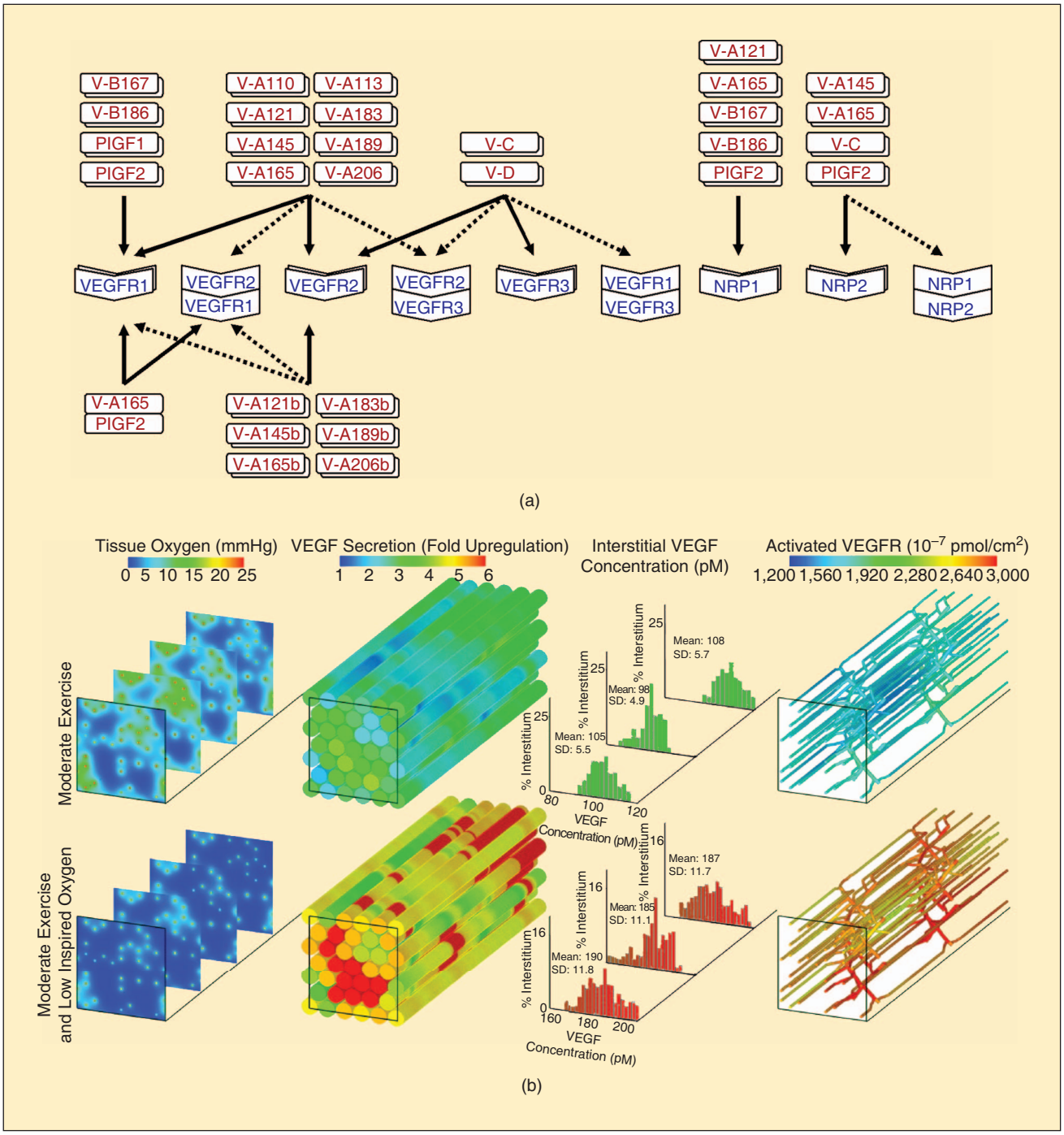

Fig. 4. Vascular endothelial growth factor and its receptors. (a) The VEGF ligandome consists of several products for each of five genes; the VEGFR receptorome comprises five genes; and their interactome is a result of the bivalency of the ligands and the homo- and heterodimerization of the monomeric cell surface receptors. Further complexity not included in this diagram includes the binding of some ligands to proteoglycans of the extracellular matrix, and the cell-released soluble truncated receptors that sequester ligands. (b) From left: The relationship between tissue oxygen levels; VEGF secretion from muscle fibers; VEGF concentration in the extracelluar (interstitial) space; and VEGF receptor activation on the microvasculature. The upper panels show moderate exercise, and the lower panels show moderate exercise with low inspired oxygen; in the two cases, the vascular networks are the same because these results represent early signaling (before vascular remodeling takes place). From left: Tissue oxygen, delivered to skeletal muscle tissue by the microvascular network; note higher oxygen near vessels; VEGF secretion from muscle fibers (cylinders), upregulated in areas of low oxygen tension because of the activation of hypoxia-inducible transcription factors; VEGF concentration in the interstitial space (between the muscle fibers), nonuniform throughout the tissue, illustrating the existence of VEGF gradients due to microanatomical heterogeneity; and lastly, distribution of VEGF receptor activation along the microvessels; note that the highest levels of activation correspond to areas of lower oxygen density, and that the activation varies more perpendicularly to the capillaries than it does along the vessels. Modified from (81).

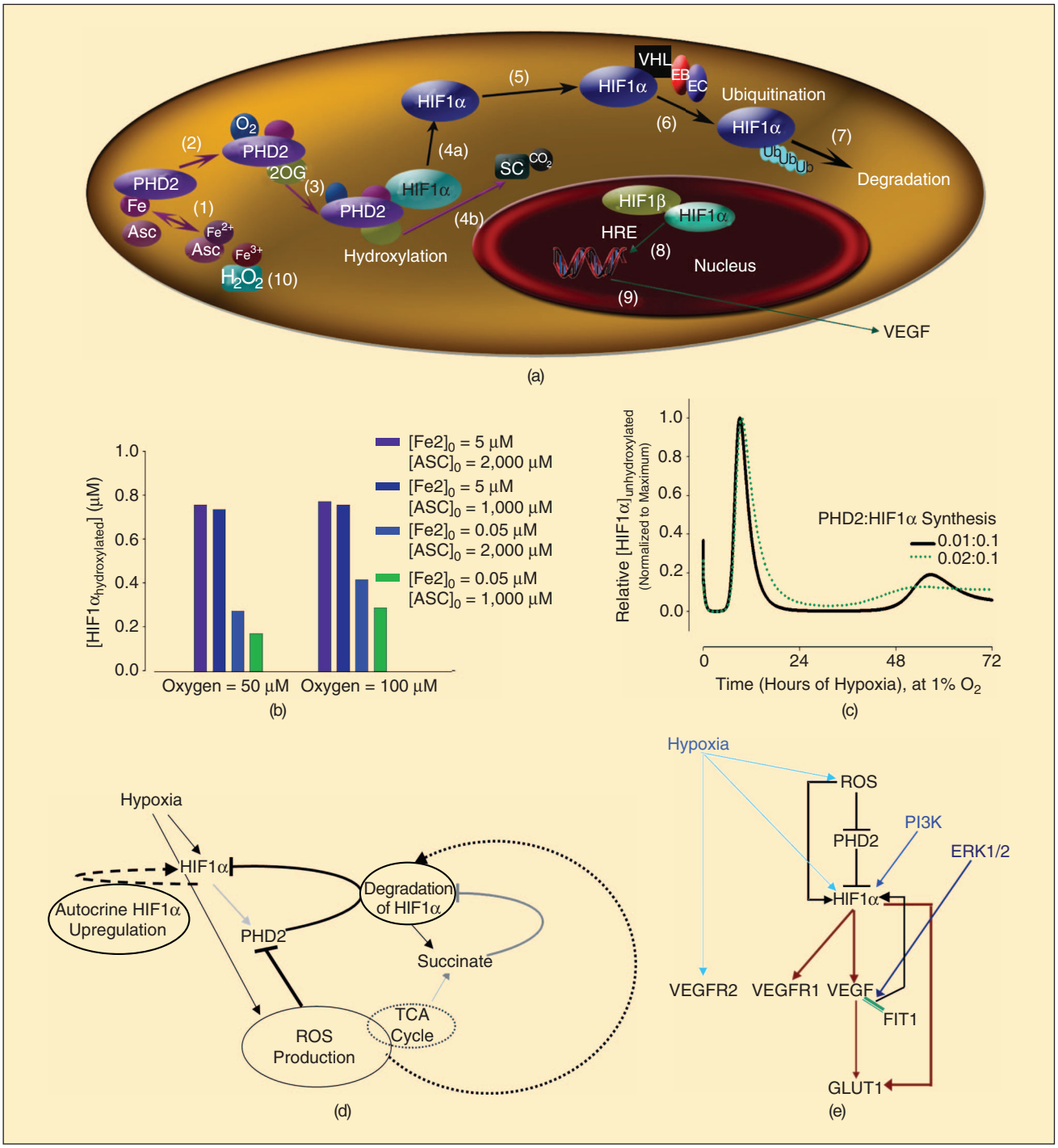

Fig. 5. Hypoxia-inducible factor 1 regulation and signaling. (a) The intracellular HIF1 pathway. HIF1α hydroxylation and degradation in the presence of oxygen involves the following: 1) the independent oxidation–reduction reactions of ascorbate (Asc) and iron (Fe); 2) and 3) prolyl hydroxlyase 2 (PHD2) binding to Fe, 2OG, and O_2; 4a) PHD2 hydroxylation of HIF1α, involving 4b) production of the by-products SC and CO_2; 5) unbound hydroxylated HIF1α moving in the cell cytoplasm; 6) the von Hippel-Lindau (VHL)–elongin B (EB)–elongin C (EC) complex ubiquitinating HIF1α; and 7) HIF1α degradation. A color change in HIF1α indicates addition of a hydroxyl group. In hypoxia, HIF1α enters the nucleus, where hydroxylation, but no degradation occurs. 8) HIF1α-HIF1β binding occurs, and 9) the HIF1 dimer can transcriptionally activate genes such as VEGF at the hypoxia response element (HRE) site. 10) ROS such as H_2O_2 also regulate HIF1. H_2O_2 binds to Fe, changing it to its Fe^{3+} form, which cannot react in HIF hydroxylation. Developed computational models have modeled the HIF1α hydroxylation process using chemical kinetics and differential equations. (b) Testing potential antiangiogenic strategies targeting HIF1α hydroxylation during normoxia and hypoxia. The effect on hydroxylation by addition of ascorbate or iron and ascrobate is shown for $[O_2] = 50$ and 100 μm. For $[Fe^{2+}] > 5\,\mu M$, the increase in hydroxylated HIF1α when $[ASc]_0$ is increased from 1,000 to 2,000 μM, remains 0.02 μM at both O_2 levels. For $[Fe^{2+}] < 5\,\mu M$, ascorbate supplementation can cause upwards of a 30% increase in HIF1α hydroxylation. For each reaction, time = 10 min. (c) Model predictions for chronic hypoxia show a secondary peak in HIF1α levels dependent on PHD2: HIF1α synthesis ratios. (d) Schematic showing the main regulators of HIF1α expression during hypoxia, as proposed by the computational model including ROS and the metabolite SC. (e) A simplified diagram illustrating stimuli modulating HIF1α in angiogenesis and HIF1 signaling effects on VEGF and VEGFRs.

change could be in the recruitment of ascorbate as a free radical scavenger, preventing ascorbate from reducing ferric iron, or preventing ascorbate from binding directly to the PHDs. If ROS increase rather than decrease free Fe^{2+}, as suggested by some experiments, HIF1α hydroxylation would instead increase [51]. Additionally, 2OG and SC are also compounds involved in HIF1α hydroxylation, whose concentrations could be altered by free radicals and mitochondrial dysfunction [50]–[52]. A fourth mechanism by which ROS could influence the HIF1 pathway is through changing the availability of oxygen to bind directly to the PHDs or changing PHD phosphorylation. To address these alternate mechanisms, and analyze possible competing factors involved in pro- and antioxidant therapy in cancer and ischemia, we developed a computational model describing the behavior of the HIF1 system in vivo and used it to predict dynamics currently inaccessible at the molecular level in vivo [Figure 5(d)] [47], [53]. Experimentally, ROS have been shown to affect the HIF1 pathway through changes in H_2O_2, Fe^{2+}, Asc, 2OG, or SC levels [50], [54], and mechanisms involving these compounds were the focus of the study. The computational model results justified the hypothesis that ROS work by two opposite ways on the HIF1 system. Results of the model predicted the following: 1) ROS up- and downregulate HIF1, through modulation of PHD2 levels; 2) ROS have a differential effect in tumor cells versus cells in ischemic conditions, leading to different apoptotic rates; and 3) cell apoptosis and H_2O_2 steady-state levels are highly dependent on extracellular H_2O_2 levels and largely independent of initial intracellular H_2O_2 levels during hypoxia.

HIF1 Intracellular Signaling to VEGF Regulation

Although HIF1 has been discussed in the context of molecular interactions so far, it is equally important to describe known and hypothesized effects of HIF1 signaling in angiogenesis. A network representation of HIF1 signaling as it relates to VEGF is shown in Figure 5(e). The schematic shows the four ways by which HIF1α protein is regulated during angiogenesis: oxygen, ROS, PHDs, and protein kinase signaling through PI3K. These four mechanisms are simplified in the diagram; multiple pathways interconnect them, which are highly dependent on the relative levels of each compound. HIF1α protein, upon being phosphorylated, enters the nucleus and binds to HIF1β. This dimer is known to bind to the hypoxic response element of VEGF, VEGFR1, and GLUT1 genes. Independently, hypoxia can upregulate VEGFR2, while the ERK1/2 pathway has recently been shown to upregulate VEGF-VEGFR1 binding and signaling, which leads to a positive feedback upregulating ERK1/2 and HIF1 [55]. There are additional feedbacks at multiple levels (not shown), including HIF1 autocrine upregulation and downregulation of HIF1 when angiogenesis effectively increases oxygen supply. Furthermore, glucose metabolism and GLUT1 are hypothesized to have effects on VEGF and its receptors, both in terms of protein concentrations and signaling. The signaling discussed so far is autocrine signaling, e.g., in a cancer cell, in a skeletal muscle during exercise, or even in ECs during angiogenesis; beyond this, there could be paracrine signaling, where, for example, VEGF levels secreted from an adjacent tissue upregulate the expression of kinases or HIF1 within ECs. Although the experimental basis for the HIF1-VEGF connectivity is being explored, computational modeling in parallel can highlight which pathways dominate under different physiological conditions and predict dynamics of the interactions during neovascularization. At the network level, a Boolean-based or thresholding approach to the kinetics, where detailed quantitative binding and signaling information is unavailable, can offer a means to characterize feedbacks. Once this signaling is well established, it would provide a critical, detailed link between molecular-based models of hypoxic sensing and VEGF secretion during angiogenesis. Having briefly mentioned this desired integration, later we summarize intricate, molecular-based models of VEGF and a multiscale simulation of VEGF distribution and signaling in skeletal muscle.

VEGF Distribution and VEGFR Activation, from Molecules to Whole Body

The VEGF family of ligands comprises five genes, each encoding multiple secreted cytokines translated via alternative splicing [56]. The expression of these genes is regulated by many microenvironmental stresses, including hypoxia [57]. The five genes encoding cell-surface VEGF receptors are three receptor tyrosine kinases (VEGFR-1, -2, and -3) and two largely nonsignaling coreceptors, neuropilin-1 and -2 [58], [59]. Neuropilins appear to increase binding of VEGF isoforms to VEGFR2 but decrease binding to VEGFR1 [60], [61]. In addition, heparan sulfate proteoglycans have been shown to be central to VEGF binding to all of these receptors [62]–[64].

Cell Signaling Is Regulated by Multiple VEGF Receptors Competing for Multiple Ligands

The VEGF receptor system transduces proliferation, migration, and survival signals. Cell behavior will therefore depend on a combination of VEGF ligand concentrations (and their receptor characteristics) and the receptor densities (e.g., VEGFR2 initiates proangiogenic signals; VEGFR1 modulates it). VEGF ligands bind multiple VEGF receptors (some simultaneously, some competitively), and receptors can bind multiple ligands (competitively, one per receptor monomer). The combination of simultaneous and competitive receptor–ligand interactions leads to complex and interesting behavior that is best studied with the aid of molecular-based computational models.

Placental growth factor (PlGF), the product of one of the VEGF genes (*plgf*) has been shown to potentiate the effects of VEGF (in this context, the products of the *vegfa* gene) on the migration, proliferation, and survival of cultured ECs [65]. Unlike VEGF, PlGF binds VEGFR1 but not VEGFR2. VEGFR1 was thought at one time not to transduce signals, largely because of its low kinase activity (relative to that of VEGFR2) [66] and the lack of developmental impact in removing the kinase domain [67]. A working hypothesis for the PlGF-VEGF synergy was formed: PlGF displaced VEGF from the dummy receptor VEGFR1, and the released VEGF bounded to VEGFR2 and increased its signaling. We constructed a computational model of in vitro cell culture experiments to simulate the binding of VEGF and PlGF to cell-surface receptors and recreate, in silico, the experiments that demonstrated synergy [65], [68]. The results of the simulations showed that under the conditions used, PlGF did displace VEGF from VEGFR1, but that no significant change in VEGFR2 binding and signaling should occur, because of the large pool of VEGF present in the cell culture media. Our alternate hypothesis for PlGF-VEGF synergy, that PlGF and VEGF cause differential signaling of VEGFR1, was experimentally validated [69]. Note that this does not preclude PlGF functioning to shift VEGF from VEGFR1 to VEGFR2 in other situations, e.g., in vivo.

The utility of computational models in testing the hypotheses of molecular mechanisms of action was further demonstrated using an expanded model of in vitro experiments that included the neuropilin-1 coreceptor and the two isoforms of VEGF, $VEGF_{121}$ and $VEGF_{165}$ [70]. These isoforms differ in their ability to bind neuropilin-1, and using seven independent sets of experiments from five groups of investigators, we validated a model of VEGF-neuropilin-VEGFR2 binding in which $VEGF_{165}$ couples (acts as a bridge for) the two receptors through distal binding sites. Neuropilin-1 had been suggested as a target for anti-VEGF therapeutics, and an antibody to neuropilin had been shown to inhibit ocular neovascularization [71]. From our simulations, it appeared that the inhibition of VEGFR2 activation caused by the antibody was beyond that possible by blocking of the neuropilin binding site for VEGF. Instead, the simulations confirmed an alternate hypothesis that matched the published data: the antibody did not interfere with VEGF-neuropilin binding but rather with the VEGFR2-neuropilin coupling, and this makes it potentially significantly more effective as a therapeutic [70], [72].

The bivalent ligand–monovalent receptor building block of the VEGF interactome [Figure 4(a)] leads to the formation of six receptor complexes (homo- and heterodimers) from the three receptor tyrosine kinases. This results in nine distinct signaling initiation points; for example, the activation of VEGFR1 by the kinase domain of VEGFR2 or of VEGFR3 results in a phosphorylation profile of VEGFR1 that is different from that resulting from activation by another VEGFR1 [73]. Moreover, the ability of different ligands to alter the activation of each receptor and the need for computational models become even more clear. Heterodimeric receptors have been shown to be important experimentally and are predicted by simulations to be prevalent [74] even to the exclusion of certain homodimers [75].

Molecular Therapies Working at the Tissue Level in VEGF-Dependent Diseases

The goal of building detailed molecular models of the VEGF and VEGF receptor families is to design and test therapeutics in silico for the many VEGF-dependent diseases. To this end, we developed a compartmental model of tissues in vivo, which includes multiple cell types (endothelial, myocytes, tumor cells) [61], [72]. For these models, microanatomical parameters are based on real tissues (e.g., surface areas of cell types; blood volume and volume of interstitial spaces). Gradients of VEGF are neglected here but simulated in other models described in the next section.

In cancer, inhibition of VEGF signaling is an active area of therapeutic research—the first anticancer VEGF therapeutics have recently been approved by the FDA, with more in the pipeline. Some of these sequester VEGF; some sequester VEGF receptors; others are small-molecule tyrosine kinase inhibitors. With our detailed molecular model of the VEGF system, we are able to make predictions of the efficacy of each of these classes of drugs. We tested three therapeutics targeting neuropilin-1: knockdown of neuropilin expression (e.g. by siRNA); peptide blockade of VEGF binding to neuropilin; and blockade of VEGFR2-neuropilin coupling [72]. The models predicted that, in tumors, the efficacy of the first two of these is heavily dependent on the microenvironment, specifically, the level of expression of the VEGF receptors VEGFR1 and VEGFR2 on the tumor vasculature. The third one was predicted to be effective in many tumors that the others were not. This impact of microenvironmental conditions (e.g., receptor expression) on therapeutic design or selection is a particularly useful direction for computational modeling. We note that, in physiological and pathological conditions, blood flow may change receptor expression and growth factor distribution in the tissue over time. In the models discussed here for integration, blood flow indirectly affects capillary structure (new sprouts, branching and vessel diameter changes are output of the cell-based module), which in turn affects receptor distribution (neuropilin, VEGFR1, and VEGFR2) and VEGF distribution in the tissue. Direct effects of blood flow (e.g., shear stress) on receptor expression or VEGF distribution are not yet considered.

Unlike cancer, ischemic diseases may be tackled using strategies to increase VEGF signaling to restore blood flow to hypoxic regions. VEGF gene and protein delivery has not so far realized its expected success in either coronary or peripheral artery disease (CAD and PAD) [76]–[78]. Exercise has been shown to be effective for some PAD patients; in exercise-trained rats, both VEGF and its receptors are upregulated, and thus we used a model of human skeletal muscle to test the impact of increasing both VEGF and its receptors compared with monotherapy of VEGF [61], [79]. The increase in VEGF results in elevated signaling by both VEGFR1 and VEGFR2, but VEGFR1 has been shown to be inhibitory or modulatory to VEGFR2's proangiogenic signaling. By increasing VEGFR2 or decreasing VEGFR1 in concert with VEGF secretion increases, the competition for VEGF binding leads to elevated proangiogenic signaling and decreased antiangiogenic signaling. This opens the possibility for using combination therapies to mimic the beneficial effects of exercise, possibly in those patients physically unable to undertake exertion.

Tissue Heterogeneity Driving Oxygen-Dependent VEGF Gradients

The 3-D structure of tissues—with some cell types secreting VEGF and others expressing the receptors that bind and internalize it—leads to the formation of gradients of the cytokine throughout the tissue. These gradients have been hypothesized to be important for the guidance of nascent angiogenic sprouts. Each tissue in the mammalian body has a different vascular structure; thus, detailed 3-D descriptions of each tissue can give insight into VEGF gradients and how these would affect vascular remodeling in that tissue.

We developed a 3-D model of the rat extensor digitorum longus muscle that includes the myocytes (muscle fibers) and the microvascular bed that supplies them with oxygen [Figure 4(b)] [18], [80]–[82]. For these simulations, VEGF secretion by the myocytes is a function of the oxygen pressure in those cells; the function is based on experimentally observed oxygen-HIF-VEGF relationships. The capillaries are not uniformly spaced within the muscle, and this leads not only to inhomogeneity of oxygen distribution but also of VEGF receptor expression within the tissue. The result is a basal level of VEGF gradients: approximately 3% change in VEGF concentration across 10 μm (representative of cellular scales) [18]. This gradient also results in a distribution of VEGFR2 activation across the many vessels in the capillary bed; that is, if a threshold of VEGFR2 signaling is required for angiogenic sprouting, some vessels can reach this threshold while others do not. The VEGF gradient is increased locally by the incorporation of cells that overexpress VEGF (cell-based therapeutics) but is not significantly

altered by the more homogenous gene therapeutic approaches to increase VEGF secretion. Exercise, which upregulates both VEGF secretion by myocytes and VEGF receptor expression on ECs, increases VEGF gradients significantly.

From Tissue Expression to Whole-Body Distribution of VEGF

VEGF is present in all tissues in the body at different levels, as well as in the blood. As indicated by an alternative name for VEGF, vascular permeability factor (VPF), it plays a role in blood-to-tissue, and hence tissue-to-tissue, communication. For normal breast tissue and skeletal muscle (the two tissues we tested computationally so far), the experimentally measured interstitial concentration of free VEGF is similar to the plasma VEGF concentration [83]–[85]. The whole-body distribution of VEGF is of particular interest in disease, e.g., in the prediction of deleterious side effects of systemic or regional VEGF-targeted therapeutics. In the light of this, we have constructed a compartment model comprising the blood compartment and normal and diseased tissue compartments [86]. Many tumors produce and release VEGF, resulting in the experimentally measured elevation of VEGF concentration in the blood [87]. This may disturb the homeostasis of other tissues, as may the systemic delivery of VEGF-sequestering agents such as bevacizumab.

Matrix Metalloproteinases

The rate-determining step for sprouting angiogenesis is the disengagement of the natural barriers that restrict the migration of the ECs into the neovascularized tissue [88]. These barriers are composed of the structural elements of the basement membrane and the ECM, comprising the different types of collagens, either fibrillar (collagen I, II) or network forming (collagen IV), elastic fibers, proteoglycans, and glycosaminoglycans (GAGs) as well as various glycoproteins, the biochemical glue for all of these components. Activated ECs overcome the ECM barrier by initiating a biochemical pathway that is responsible for the proteolytic degradation of the structural elements. Growth factor stimulation of the ECs leads to the secretion of ECM-digesting enzymes. Proteases are also secreted by stromal cells and, in the case of tumor angiogenesis, by the surrounding tumor cells. The production of proteases by these cells can lead to profound changes in the structure of the surrounding ECM, reducing the restrictive structural barriers and allowing the migrating and proliferating ECs to penetrate avascular or poorly vascularized tissue [66], [89], [90]. One well-studied class of proteases is the MMPs.

MMPs: Regulation and Function

MMPs have been recognized as important proteolytic modulators of the ECM, cytokines such as VEGF and bFGF [91]–[95], cell-adhesion molecules, and signaling receptors [96]–[98]. Collectively, MMPs regulate the angiogenic processes of sprout initiation [99], tube formation and stability [100], and capillary regression. There are more than 25 known MMPs, which can be subdivided based on their substrate specificity as either collagenases (e.g., MMPs 1, 8, 13, MT1-MMP), gelatinases (MMPs 2, 9), or stromelysins (MMPs 3, 10, 11), and by cellular localization as either diffusible or membrane-type MMPs (MT-MMPs) [101].

In response to various angiogenic factors, both the expression and activation of MMPs are upregulated, and MMPs are localized at the fronts of migrating cells. Because most of the diffusible MMP species are synthesized as inactive zymogens, with latency maintained by the enzymes' propeptide domains, proteolytic activity usually requires a bimolecular or multimolecular proteolytic processing event in which the MMP prodomain is cleaved [101]. This proteolytic event can be carried out by another, already-activated protease from the serine protease or MMP families. The activation of MT1-MMP occurs intracellularly at the trans-Golgi network. Among the six MT-MMPs, MT1-MMP was the first to be identified as a specific activator of pro-MMP2 (gelatinase A) at the cell surface [102]. Activators of pro-MMP9 include active MMPs 2, 3, and 13, as well as the serine protease plasmin [103].

Additional regulation of MMP activity is mediated by the tissue inhibitors of metalloproteinases (TIMPs), of which four have been discovered (TIMP 1–4) [104]. TIMPs are a family of low-molecular-weight (20–30 kDa) proteins that bind primarily to the active forms of MMPs, both surface localized and diffusible, and inhibit their proteolytic activity. Despite their inhibitory role for active MMPs, TIMPs may assist in pro-MMP activation by facilitating the interaction between a pro-MMP and an active MMP. For example, TIMP2 can act both as an activating adaptor for pro-MMP2 and as an inhibitor of MT1-MMP. In order for pro-MMP2 to be activated, it must bind to a preformed MT1-MMP:TIMP2 complex, which acts as a cell-surface receptor for pro-MMP2 [105]. Specifically, the active amino-terminal domain of TIMP2 binds and inhibits the catalytic domain of a free MT1-MMP, while the carboxy-terminal domain of TIMP2 binds to the PEX domain of pro-MMP2. Once pro-MMP2 is bound at the cell surface, a second TIMP2-free MT1-MMP molecule serves as the activator and cleaves the propeptide of pro-MMP2 in the receptor complex [105]. The requisite proximity of receptor and activator molecules is accomplished by forming a homophilic complex involving the PEX domain of MT1-MMP [106]. Because of TIMP2's dual role, increasing TIMP2 expression does not directly correlate with inhibiting protease activity [107]. TIMP2 effectively plays a role in modulating the localization of proteolytic activity from the cellular surface (MT1-MMP) to the diffusible forms (MMP2) [108]. These processes are illustrated in Figure 6. It is important to note that TIMPs may also have MMP-independent functions. For example, TIMP3 is known to directly interfere with the binding of VEGF to VEGFR2.

Models of MMPs, Cell Migration, and VEGF Release

To delineate the ECM-digesting biochemical pathways as well as the molecular mechanisms involved and the nature of signaling during the cell migration, numerous in vitro systems mimicking the natural tissue environment have been developed. These include the growth of ECs or blood vessel fragments on two-dimensional (2-D) membranes, in 3-D type I collagen matrices, and in Matrigel [109]. A common characteristic of these in vitro systems is not only EC migration but also the subsequent remodeling of the surrounding ECM. The role of MMPs in sprout formation has also been directly observed in vivo, for example, in ischemia-induced angiogenesis in rat skeletal muscle [99]. The role of basement membrane penetration is primarily attributed to the type IV collagenases (MMP2 and MMP9), while the proteolysis of type I collagen in the ECM can be performed by any of the fibrillar collagenases, MT1-MMP or MMP2. Despite a volume of experimental data [66], [105], [110]–[113], there is no integrated, quantitative model of ECM degradation and

release of growth factors from the ECM. Our goal is to initiate this effort (Figure 6). In our computational approach to study angiogenesis, MMPs operate at the molecular scale to determine cellular level decisions and events. We have formulated biochemically detailed models of the postsecretional regulation of MMPs, including the processes of proMMP activation and MMP inhibition by the TIMPs [107], [108], [114]. Our kinetic models capture the dynamics of the MT1-MMP/TIMP2/proMMP2 and MMP3/TIMP1/proMMP9 systems under in vitro conditions [107], [114]. These models reproduce the biphasic dependence of MMP2 activation on TIMP2 as well as biphasic inhibition of MMP9 by TIMP1. These local models are incorporated into cell-level geometrical models and MMP secretion to provide spatial and temporal descriptions of collagen proteolysis in the vicinity of a moving tip cell. The MT1-MMP/TIMP2/proMMP2 system has the ability to describe both the EC penetration of the basement membrane as well as migration within the ECM. Ultimately, this information is fed back to the cellular compartment to determine the migratory response of a sprout.

Currently, we are also developing models to recapitulate the observed role of MMPs in the release of VEGF. VEGF can bind to heparan sulfate GAGs in the basement membrane and ECM proteoglycans. This pool of VEGF is biologically relevant as it has been shown to be active either in its bound form or as soluble molecules via proteolytic cleavage of VEGF or the proteoglycan core proteins [115]. MMPs that can directly cleave VEGF include MMPs -1, -3, -7, -9, -16, and -19 [94]. The proteolysis results in a VEGF isoform of 113 amino acids, which exhibits similar angiogenic behavior to the $VEGF_{121}$ isoform [94]. The released VEGF regulates the sprout patterns and the resulting efficacy of tissue oxygenation, possibly as a result of MMPs modulating both the mean levels of VEGF in tissue as well as the VEGF spatial distribution.

Our kinetic models of MMP reaction networks tie into our existing cell-level models to describe the role MMPs play in sprout migration during angiogenesis. In a commonly accepted paradigm of sprouting angiogenesis, secreted VEGF induced by hypoxia activates ECs to migrate up a VEGF gradient into regions of hypoxic tissue to enable blood flow and restore oxygen tension. MMPs are secreted by the ECs to locally degrade the natural barriers that the activated cells face. Proteases can also be derived from stromal, tumor, and leukocytes such as neutrophils or macrophages [116]. The migration of individual sprouts may thus be mediated by nonspecific events in the tissues undergoing angiogenesis as well as localized sprout-dependent proteolysis.

Given the current in vitro and in vivo experimental data, the exact role and cellular origin of MMPs (as well as their substrates and end effects) under various physiological and pathological angiogenic processes are not completely understood. In muscle angiogenesis, MMPs are highly regulated by the nature of the mechanical stimuli and the nature of the induced ischemia. For example, exercise-induced ischemia seems to have different characteristics than injury-induced or artery ligation-induced ischemia. Specifically, in the latter, the presence of an inflammatory response may result in the migration of neutrophils and macrophages; these cells secrete MMPs

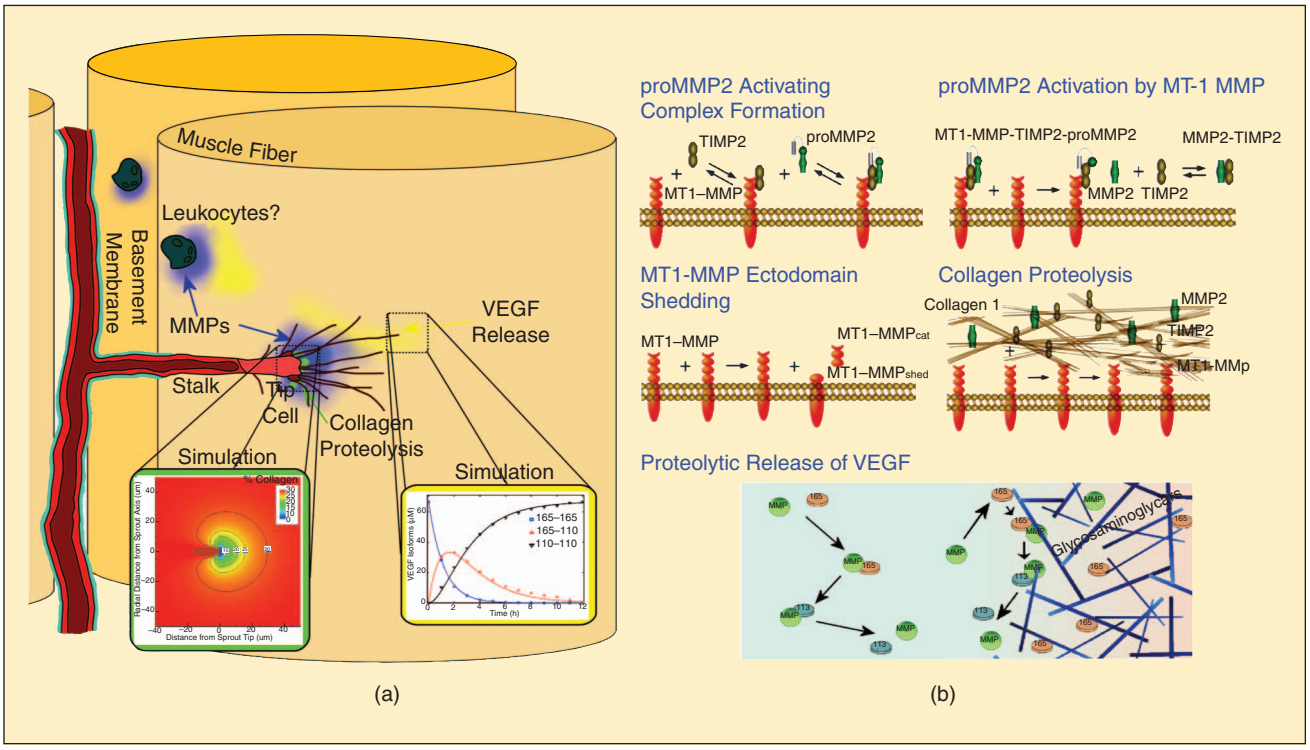

Fig. 6. Schematic showing the existing integrative MMP models, as applied to skeletal muscle angiogenesis. (a) Illustration of the role of MMPs in sprouting angiogenesis. MMPs are involved in both the proteolysis of the structural components in the extracellular matrix such as type I collagen as well as the release of VEGF bound to GAG chains in the heparan sulfate proteoglycans. (b) Schematic of MMP reactions involved in collagen proteolysis and VEGF release. Collagen proteolysis is conducted by both MT1-MMP and MMP2, the latter which is also activated by MT1-MMP in the presence of TIMP2. VEGF release as a 113 amino-acid isoform can be performed by MMPs 1, 3, 7, 9, 16, 19, some or all of which may play a role in angiogenesis.

and thereby alter the release of VEGF from the ECM [116]. The nonmigratory role of MMPs is much more complex and uncertain than the role of MMPs in cell migration. Just as MMPs can regulate VEGF levels in tissue, the products of MMP-mediated proteolysis, e.g., angiostatin, can have an antiangiogenic role [116]. Thus, the nonmigratory role of MMPs is tissue- and situation-specific. Here, the role of MMPs is deeply interconnected with that of its substrates, both in terms of temporal and spatial localization.

Capillary Sprout Formation

The interaction of VEGF, MMPs, and the ECM at the molecular level set the stage for a series of events that can be described at the cell level: tip cell activation; EC migration, chemotaxis and haptotaxis, and cell proliferation. We used an agent-based approach to develop a 3-D, computational model to mimic cellular sprouting at the onset of angiogenesis [Figure 7(a)] [117]. The model is based on experimental work compiled from extensive literature research and applied to conditions that might occur in a 3-D in vitro setting. We represent physiological changes at the cell level; simulate in 3-D assumptions behind cell activation, migration, elongation, proliferation, and branching; and test cell level behavior in response to different stimuli, focusing in the current model on activation by a threshold change in VEGF and changes in Dll4 ligand presence. Individual cell behaviors (activation, elongation, migration, and proliferation) combine to produce a novel capillary network, emerging out of combinatorially complex interactions of single cells. Capillary formation can then be characterized by branching, multiple sprouts, anastomosis, looping, and regression. The cell-based model presents a 3-D framework upon which the hypotheses about the tissue-level effect of molecular and cell-level events are tested and quantitatively characterize possible mechanisms underlying blood vessel growth. A particular benefit of the rule- or event-based modeling is the ability to easily produce in silico knockouts on multiple biological levels. Here we give two examples—a molecular level knockout of Dll4 ligand [Figure 7(b)] and cell-level knockouts of individual cellular processes [Figure 7(c)]—and show their predicted effects on vessel growth.

Dll4 Knockouts

Dll4 is a transmembrane ligand for notch receptors, and it is critical to vascular development. So important is Dll4, that like VEGF, haploinsufficiency of the Dll4 gene is embryonically lethal in many mouse strains as a result of extensive vascular defects [118]–[120]. Dll4 is primarily expressed in ECs, and it is correlated to the local concentration of VEGF [121] as well as to VEGF receptor concentrations; a blockade of VEGF leads to a decrease of Dll4 [122], while notch-delta signaling downregulates VEGFR2 [123]. One study showed that the presence of Dll4 reduced tip cell formation as a function of VEGF [124], and another demonstrated that notch suppressed branching and proliferation at the sprout tip [118]. A Dll4 deficiency causes an increase in sprout formation, but the vessels appear nonproductive, with less capability of carrying blood or reducing hypoxia in surrounding tissue [122]. Overexpression of Dll4 diminishes the growth of new sprout tips. In the computational research presented here, we show the effects of VEGF protein concentrations and Dll4 haploinsufficiency on ECs. We show how cell-level behavior alone contributes to differences in capillary network formation, and the vascular networks resulting from the model appear similar in vascular density, branching, and tortuosity to networks found in Dll4 experiments [Figure 7(b)].

Event Knockouts

Using the computational model, in silico experiments were performed that knocked-out events of migration, elongation or proliferation of tip or stalk cells independently. The goal was to test the contribution of each cellular parameter at the total vessel level. Cumulative vessel length changes over time were used for quantitative comparisons of the effect on capillary growth. Results from the model are shown in Figure 7(c). In silico experiment 4 is the positive control where all events are allowed, while experiment 3 is the negative control, where there is no migration, elongation, or proliferation beyond the initial activation and tip cell formation. Cell elongation had a significant effect on total vessel length [experiment 2, Figure 7(c)]. A hypothesis of the model at the cellular level is that tip elongation is the stimulus for cell proliferation and migration; without it, the cell may migrate to an extent, but will not proliferate until stimulated. The event knockout in silico experiments also predict that stalk cell proliferation dominates tip cell proliferation in determining total vessel growth, even during the first 24 h of angiogenesis; experiment 1 is the in silico experiment where tip cell proliferation is inhibited [Figure 7(c)].

Integration

The utility of the molecular-cell-level, rule-based model will grow, as models are integrated within it, and the detailed rules based on experiments are applied to specific in vivo physiological conditions. Beyond mere integration, the goal is to coordinate feedbacks (Figure 2). From blood flow to oxygen transport, to hypoxic response to VEGF secretion, to cell sensing, proteolyzing its matrix and migrating through the ECM to cell proliferation, sprout formation and new vessel building, and back. The first example of this integration includes four models [Figure 2(b)]. An initial vascular network structure in 3-D is input into the model. This structure can be obtained through quantitative descriptions or image analysis of microvessels in specific tissues, such as muscle or tumor. Blood flow and hematocrit throughout the vessels are then calculated, which leads to prediction of oxygen distribution. Using an empirical correlation between oxygen pressure, HIF activation, and local VEGF secretion levels, we can then predict the VEGF distribution in the tissue. VEGF concentrations and gradients are in turn sensed by the ECs, and vessel growth can result from cellular migration and proliferation. The formation of new microvasculature starts the next round of calculations, and the new structure is fed back into the blood-flow module.

Although this whole process could (and has) be modeled at some level, the integration of the detailed molecular level components with cellular resolution and network response modules is what will make computational angiogenesis models powerful tools in understanding the complex biology and dynamics of neovascularization. A goal is to coordinate angiogenic models written in diverse languages and apply the process to dynamically represent healthy and diseased vascular conditions. The technical part of the integration revolves around a unit called the controller, which provides the integration platform. The controller can link with the modules written in different languages and pass the parameters between them. Parameters include kinetics (e.g., rate constants) and physical

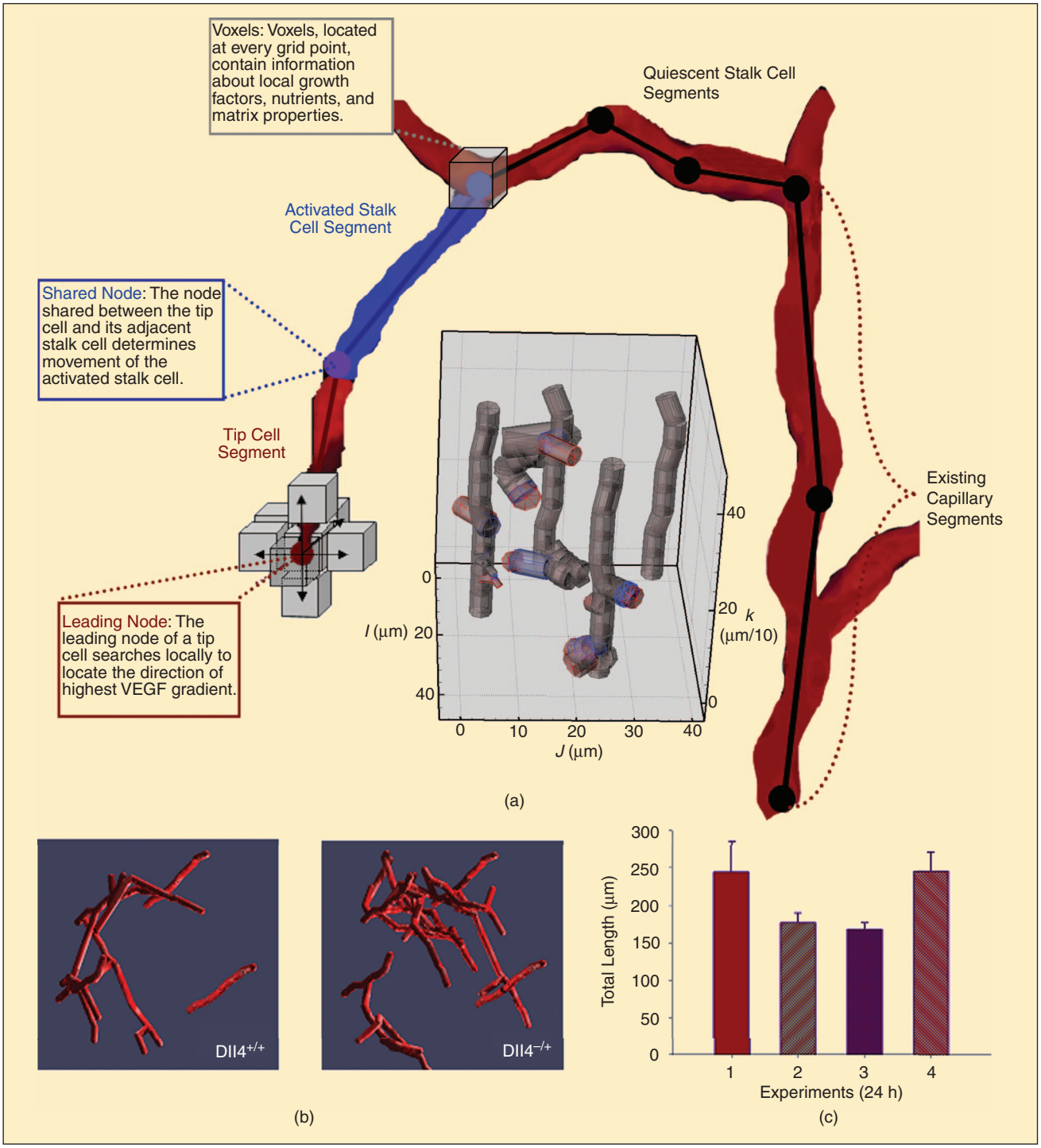

Fig. 7. Schematics and figures for the cell-based module. (a) Schematic of the three-dimensional cell-based module. Capillaries are represented by ECs. An example of a growing network with four capillaries is shown in the gray inset. Cells are divided into segments. Each segment is represented by two nodes. Currently, cell segments are modeled as cylinders specified by a length and radius (gray inset); an activated segment's length and radius can change during a model run. The local environment surrounding a cell is defined in each voxel of the grid. In the present model, voxels contain values for the local VEGF concentration. All cell segments have the capability of sensing what is located in the 26 voxels surrounding each of its nodes. For every time step of the current model, this sensing is restricted to the leading node of the tip cell (red) and the adjacent node (purple), shared by the tip and activated stalk segment. The local search for the highest growth factor gradient surrounding the leading node of a tip cell determines the direction the sprout tip moves. (b) Effect of haploinsufficiency of Dll4 on blood vessel sprouting compared with control conditions, shown by visual output of the model. In the Dll4 knockout, total vessel length is greater, while the vessels are more tortuous. (c) Vessel length predicted by the model, 24 h after the onset of sprouting angiogenesis, for different in silico experiments using event knockouts. Experiment 4 is the positive control, where all processes are allowed. Experiment 3 is the negative control, where only activation of tip cells occurs. Experiment 2 is where cell elongation is restricted and all other processes are allowed; and in experiment 1, there is no tip cell proliferation, while all other events occur.

parameters (e.g., 3-D tissue and vascular geometry). To minimize error propagation during integration, parameter sensitivity analysis and potentially parameter optimization will be performed on the individual models and independently on the combined integrated model. The models will be validated for their specific application with available tissue-, cell- and molecular-specific literature data, and the output of the integrated model will be compared to experiments. This multiscale integration allows predictions across both temporal and spatial scales, through an interactive hierarchy of molecular, cellular, tissue, and organ system components, which is essential in rational drug development and therapeutic regime design, and will encourage numerous related experiments in parallel.

Perspectives and Conclusions

Computational models of angiogenesis so far have brought us a critical quantitative perspective on the field, predicted cell migratory speeds through different tissue matrices [108], allowed in silico predictions of molecular- and cell-based therapies [18], [40], [82], differentiated vasculature from cancerous brain tissue from noncancerous sites [125], among other contributions.

Multiscale molecular-based modeling paves the way to a future for integrative medicine, where we will be able to prescribe optimal individualized therapeutic or nutritional regimes based on molecular biomarkers for individual patients—for example, using a blood sample or biopsy to computationally predict whole body response. To reach this point, we need highly evolved integration of models. This article aims to promote integration of angiogenesis-related models, both integration of the work described herein and many other existing and future models. With this in mind, we conclude by highlighting tools for multiscale modeling (Table 2) and briefly mentioning areas for integrative, multiscale modeling that merit in-depth discussion, but are limited to a few words and citations in this paper: parameter sweeping, experimental comparisons, and learning algorithms.

Dealing with a large number of parameters, some, if not most, unknown experimentally, comes with the process of multiscale, integrative modeling. Sophisticated, accurate, efficient means of analyzing parameter sensitivity are starting to be applied in biological models and will likely become a requirement for rapid progress in the field, as well as a means to help correlate model parameters with experiments. Learning algorithms, too, limited in their application to the field of angiogenesis, will likely emerge as a means to keep programs updated and evolving as new data become available—moreover, such algorithms could be employed to better portray biological adaptation to disease states and angiogenic development, as examples.

Table 2. Tools relevant to multiscale angiogenesis models.

	Multiscale Modeling Tools
Integrated databases (outside NCBI)	Cell cycle database (136) BRENDA enzyme database (137) CellX cell type-specific training set database (138) BioXBase (139) Pathway databases (140), (141)
Markup languages and formalisms	SBML (142) CellML, AnatML, FieldML (143) Sequential logic formalism (144) Kohn maps (145)
Parameter analysis and model comparisons	Sloppy parameter sensitivities (146) Parameter extrapolation: metabolic network to dynamics (147) Latin hypercube sampling in multiscale models (148) Design optimization of network simulations, with parameter estimation (149)
Integrators and integration	Comparison of integration tools (150) Functional interactions in signaling and gene networks (151)
Computational resources	MIRIAM modeling annotation (152) Systematic clinical-computational model integration (153) SigPath information system (154) JSim simulation interface Quantitative cellular imaging review (155) Gepasi biochemical simulation software (156)
Integrative multiscale projects relevant to angiogenesis	IntBioSim: http://intbiosim.org/ Integrative Biology Project: http://www.integrativebiology.ox.ac.uk/ Physiome Project (12): http://www.physiome.org.nz/, http://www.physiome.org/ E-Cell Project (157): http://www.e-cell.org/ecell/ MSM Wiki: http://www.imagwiki.org/mediawiki/ VIABS Project: http://www.beaconprojects.org.uk/viabs.htm Virtual Cell Project (158): http://www.vcell.org/ CompuCell3D (159): http://compucell3d.org
Recent reviews: multiscale and integration	(11), (12), (160)–(166)

Matrix metalloproteinases are the key molecules involved in the process of an activated cell proteolyzing its surrounding extracellular matrix.

Integration of molecular mechanisms with cell- and organ-level models allows investigators to study angiogenesis from perspectives in time and space that were once unattainable. As new tools develop for the systematic validation, integration, visualization, and adaptation of these models, the field of angiogenesis heralds an era where modeling becomes an essential component of rigorous experimental design and therapeutic advances.

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Amina A. Qutub graduated with a B.S. degree in chemical engineering from Rice University in 1999. She received her Ph.D. degree in bioengineering from the University of California, Berkeley, and the University of California, San Francisco (UCSF), in 2004, under the mentorship of C. Anthony Hunt. For her doctoral work, she researched the blood–brain barrier, using computational and experimental approaches. She is a postdoctoral fellow in biomedical engineering at Johns Hopkins University School of Medicine, under the mentorship of Aleksander S. Popel. Her research focuses on modeling hypoxic response and sprouting angiogenesis.

Feilim Mac Gabhann received a B.E. degree in chemical engineering at University College Dublin in 1997. He received his Ph.D. degree in biomedical engineering at Johns Hopkins University in 2007, under the mentorship of Aleksander S. Popel. His thesis work focused on computational modeling of vascular endothelial growth factor and its receptors. He is a postdoctoral fellow at the University of Virginia Robert M. Berne Cardiovascular Research Center, under the mentorship of Shayn M. Peirce-Cottler and Thomas C. Skalak, where he is working on experimental studies of angiogenesis and arteriogenesis.

Emmanouil D. Karagiannis received his B.E. degree in chemical engineering at Aristotle University of Thessaloniki, Greece in 2001. He received his Ph.D. degree in biomedical engineering at Johns Hopkins University in 2008, under the mentorship of Aleksander S. Popel. He is a postdoctoral fellow in chemical engineering at Massachusetts Institute of Technology, under the mentorship of Robert S. Langer. His primary research interests include the combination of computational and high-throughput screening experimental methodologies for the identification of bioactive peptides.

Prakash Vempati received his B.S. degree in biomedical engineering at Johns Hopkins University, where he started work under Aleksander S. Popel, characterizing the role of matrix metalloproteinase 9. He is an M.S. candidate in biomedical engineering at Johns Hopkins University School of Medicine, under the mentorship of Aleksander S. Popel. His research focuses on the kinetic characterization of matrix metalloproteinase systems as well as their interactions with vascular endothelial growth factor to elucidate their roles in angiogenesis.

Aleksander S. Popel is a professor of biomedical engineering at Johns Hopkins University School of Medicine. His research interests are in systems and computational biology, microcirculation and angiogenesis. He is a fellow of the American Institute of Medical and Biological Engineering, American Heart Association, American Physiological Society, and American Society of Mechanical Engineers, and an inaugural fellow of the Biomedical Engineering Society. He has been a member of several editorial and review boards of biological and biomedical engineering journals, such as *Annals of Biomedical Engineering, American Journal of Physiology, Microcirculation, Microvascular Research*, and *Cellular and Molecular Bioengineering*. He is cochair of the Physiome and Bioengineering Committee of the International Union of Physiologica.

Address for Correspondence: Amina A. Qutub, Department of Biomedical Engineering, School of Medicine, Johns Hopkins University, 613 Traylor Building, 720 Rutland Avenue, Baltimore, MD 21205 USA. E-mail: aqutub@jhu.edu.

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