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Jamie A. Davies

Branching Morphogenesis

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Branching Morphogenesis

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To all members of our laboratories,
past and present, in gratitude for their inspiration,
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CHAPTER 1

Why a Book on Branching, and Why Now?

Jamie A. Davies

In a world overloaded with information, in which university library shelves bend under the weight of worthy tomes and the number of journals has been doubling every fifteen years,[1,2] a prospective reader is fully entitled to eye any new text suspiciously and to ask whether there is really a need for yet another book. The question is always a valid one, and was perhaps summed up most clearly by the reviewer who remarked of a manuscript under his scrutiny that '*this paper fills a much needed gap in the literature*'.* It is therefore a duty of any author or editor to begin the introduction of a new work with a justification for its existence.

For this particular volume, providing such a justification is easy. The subject matter is of critical importance to our understanding of the normal development of animals and plants and is a necessary component in the emerging technology of tissue engineering. Study of branching is changing quickly and is expanding through new links between cell biology and mathematical modelling. Most critically of all, its subject material has traditionally been scattered through the texts and journals of many different disciplines and has not been brought together in one place before. The recent emergence of general principles behind branching morphogenesis, and the observation that apparently disparate systems seem to share deep biological similarities,[4] is a strong and timely reason for considering them together, now, in a single volume in which each chapter is contributed by a world expert in a particular field.

Branching Morphogenesis Is Important and Pervasive

The development of repeatedly-branched structures is an important mechanism of morphogenesis across a wide range of phyla and scales. In some organisms, such as trees, branching shapes the complete body plan and is their most obvious morphological attribute. Most plants and multicellular fungi share this property, although in the case of fungi the branched structures are very fine and, to the naked eye, are not as obvious as unbranched reproductive structures such as mushrooms. Some animals also have a branched body plan but, in most phyla, branching is hidden away in the internal anatomy and is not obvious from external form. We are examples of such creatures, having unbranched exteriors but having insides riddled with interlinked networks of branched endothelial and epithelial tubes.

Branching usually arises where there is a reason to maximise the total area of contact between a structure and the environment that surrounds it, particularly where there is also a reason to pack this area of contact in a small volume (that is, an organism gains some functional selective advantage by doing this). For plant shoot systems, the 'aim' is to maximise the area for light capture and gas exchange: while large areas could be produced by the growth of a single enormous leaf, mechanical constraints (gravity, wind damage etc) limit this strategy to

* A history of this acerbic phrase has been reviewed elsewhere,[3] but so cliché d has it become that some reviewers now seem to miss its precise meaning, and use it even in very positive reviews: type the phrase into a web search engine to find many examples.

Branching Morphogenesis, edited by Jamie A. Davies.

very small plants or those supported by, for example, floating on water; self-supporting large plants are forced to use branched structures. Similarly, plant root systems need to achieve large areas of contact between themselves and the soil, an important source of water and minerals, and the production of fine branches has the added advantage that they can penetrate between particles of soil and thus expand into an almost 'solid' environment.

In animal tissues, branching is normally used to pack a large surface area for exchange between the external environment and internal tissues, or between two internal 'compartments', into a small volume. The branched structures of mammalian lungs are an example and here, as in most other systems, the branching tubes are not themselves the main surfaces over which substance exchange takes place. Rather, gas exchange takes place in specialised air sacs, alveoli, that appear at the ends of the finest branches; the branching system itself is simply a means of connecting many alveoli to the outside world while minimising the total distance from each to the final exit from the body (an alternative design, connecting all of the alveoli to a single long tube, would suffer the disadvantage that the most distant alveoli would be able to exchange gases with the outside only very inefficiently). A range of other branched epithelia act as 'drains' for substances (saliva, urine, seminal fluid, tears, milk etc) produced in specialised terminal structures, sometimes also called alveoli.

The blood and lymphatic systems, based on endothelia, are specialized for exchange of substances between body compartments (the 'tissues' and 'the circulation', each of which really constitutes several different functional compartments in itself). The aim is to ensure that no part of the tissue is more than a short distance from a blood vessel, and arterial flows ramify ever more finely in tissues to achieve this. In the case of vertebrate blood systems, the finest branches then connect with fine branches of a venous system, collecting post-exchange blood and draining it to successively larger-bore vessels to return it to the heart (in some organs, such as kidney and gut, blood is collected from the arterial system by an intermediate branching system that takes it to a second set of capillaries before it returns to the venous system proper, but such complications are beyond the scope of this introductory chapter).

The means for physical substance exchange is not the only system that has to spread throughout the body; there is also the need for distribution of 'command and control' information. Some of this is achieved using exchange of signalling molecules (hormones) between tissues and the general circulation but much of it is achieved by connecting specific tissue elements via nerves. In simple body plans such as those of cnidaria (sea anemonies, jellyfish etc), this is achieved by a distributed nerve net. In more complex animals it is done by connection of tissues to a central information processing unit—a ganglion or a brain. Nerves, which are bundles of neuronal cell processes (axons), run from central nervous system out to the tissues where they divide increasingly finely and eventually single axons may branch to make connections with multiple targets, for example, muscle fibres. Within the central nervous system, highly branched systems of neuronal processes are used to collect and integrate signals from multiple input neurons. In a clear reference to their shape, these are called dendritic arbours (from Greek *dendros,* = branch, and Latin *arbor,* = tree: US English retains the Latin spelling).

Branching morphogenesis produces structures on scales ranging from micrometres (the processes of a single cell) through centimeters (branching epithelia in mammalian organs) to tens of metres (trees). Indeed, the current record holder for the world's largest organism is a woodland fungus, *Armillaria ostoyaei,* which is over 2000 years old and spans about 10 square kilometers of forest floor;[5] it is composed almost entirely of a huge network of branched hyphae.

Branching morphogenesis is therefore an important and pervasive mechanism of development.

Patterns of Branching

All branched biological structures are generated by variations on just a few general mechanisms. One very common mechanism is branching of an elongated structure, such as a plant stem, an epithelial tubule or a cell process, by division of its growing tip into two or more new

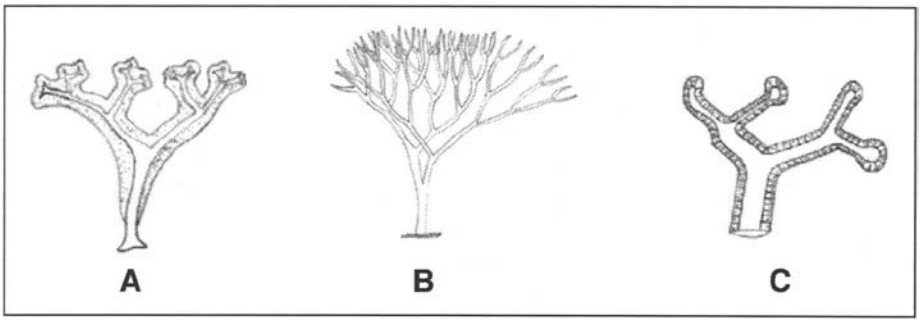

Figure 1. Examples of dipodial branching, in A) a green alga of the *Fucus* genus, B) a red alga of the *Polyides* genus and C) in the developing airway epithelium of a mouse embryo.

tips (Fig. 1). Variations on this theme certainly exist, both in terms of the numbers of tips formed and the method by which the tips divide, but the general process accounts for a great deal of branching morphogenesis over a huge range of scales. In its simplest form—dipodial branching—the daughters of each branching event are 'equal' and no one branch dominates the structure. A common variation is monopodial branching (Fig. 2), in which secondary branches form from one dominant stalk. This pattern is obvious in many trees, but is also found in animal tissues such as the mammary gland. In most examples of monopodial branching, the dominant stalk develops first and the side branches appear as later additions.

A very different mechanism for generating branched tubes is to divide up a large tube into many smaller ones by the introduction of longitudinal barriers (Fig. 3). This process—intussusceptive branching—creates a much larger surface area over which exchange can take place between the fluid in the tubes and their surroundings. For this reason, and also because it is well-adapted for tubes that form part of a circulation system rather than having closed ends,

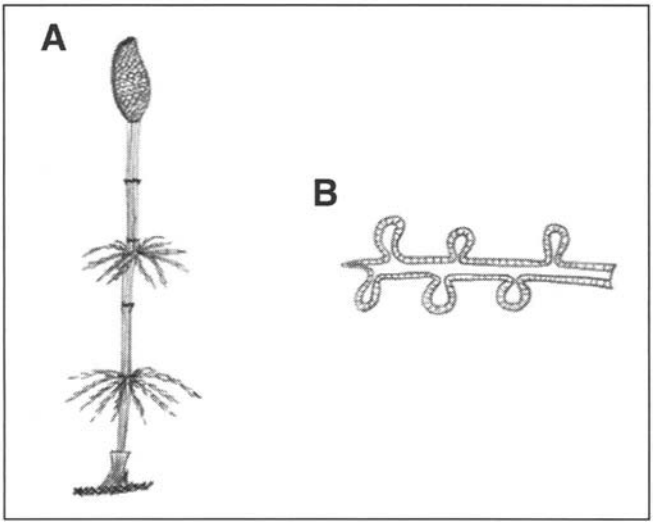

Figure 2. Examples of monopodial branching, in A) the horsetail *Equisetum* and B) mouse mammarsy epithelium budding alveoli during pregnancy.

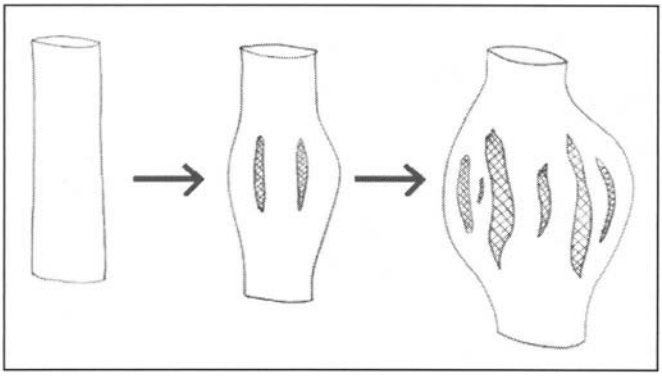

Figure 3. Intussusceptive branching, by the invasion of a vessel space by other tissue (following infolding of the vessel wall). The longitudinal dimension of this diagram has been compressed for clarity.

intussusceptive branching is common in the development of blood vessels. It is also how most river deltas form.

The above mechanisms fall squarely into the category of 'branching morphogenesis' because they operate by division of one thing into many. There are other ways of making branched structures which are not normally considered *bona fide* examples of branching morphogenesis but which are worth mentioning in this introductory chapter, if only because they are not considered elsewhere in this book. One is related to intussusceptive branching, and consists of producing a 'branched' gross structure by deletion of cell populations: an example is the 'branched' structure of chicken feet, which arises, in part from apoptosis of the cells that would otherwise form a continuous web between the toes[6] (Fig. 4). The other fairly common mechanism for creating a branched biological structure is the fusion of elements that originate separately and then converge. An example of branching by convergence is seen in the aggregation of myxamoebae of *Dictyostelium discoideum*, in which migratory cells form streams that join together and converge on one point[7] (Fig. 5). Another is seen in the mesonephros (temporary kidney) of mammalian embryos, in which tubules form independently but converge on to a common duct.[8]

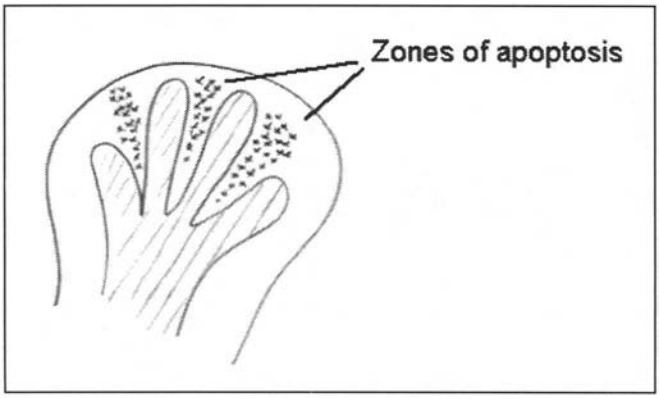

Figure 4. The role of apoptosis in separating the "branches" of the foot (the toes).

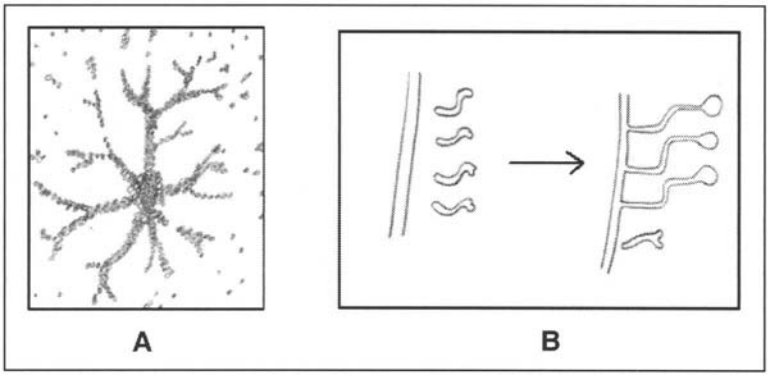

Figure 5. Formation of branched structures by convergence, in A) aggregating *Dictyostelium* myxamoebae, and B) mensonephric tubules joining a common nephric duct in mammalian embryogenesis.

Research into Branching

Branching morphogenesis can be studied at many different levels from molecular genetics to mathematical modelling. Some of the earliest work on branched systems was mathematical; Leornardo da Vinci studied the dimensions of trees that develop by dipodial branching, and showed that the ratio of the diameter of a branch of generation n to the diameter of one of generation $n+1$ was constant for all n (Fig. 6). The constancy of this number, now called da Vinci's number,[9] implied that such structures are scale-free (one cannot deduce, from the ratio of branch sizes alone, whether the branches in question are the very largest or the very finest) and da Vinci's work on branching was one of the earliest examples of what would now be considered the mathematics of fractals. More recently, similar analyses have been performed on branched structures in animals, for example canine airway epithelium,[10] with the result that da Vinci's rule holds for a large range of n. It is clear, though, that the very first branching events

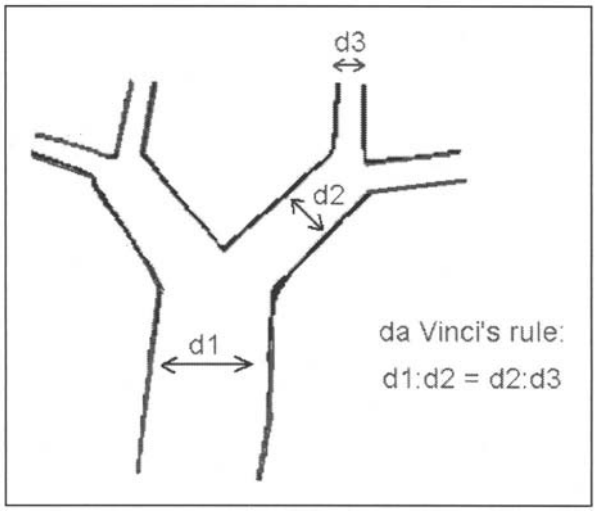

Figure 6. Leonardo da Vinci's rule.

and the very last depart from the rule in most systems, and fractal geometry is only a useful approximation for the middle stages of growth. Even then, additional systems must operate to set up the characteristic shape of each organ and to avoid collisions.[11]

Fractal studies have been joined by mathematical approaches based on the characteristics of purely physical models, in an attempt to understand the extent to which biological branching morphogenesis might rely on gross physical properties of their components, such as viscosity, pressure and mechanical stress, rather than on any especially 'biological' characteristics such as cytoskeletal remodelling. Some of the most interesting of these models have used 'viscous fingering'—the branching phenomenon that takes place when a liquid of low viscosity is forced into one of higher viscosity—to model epithelial branching in animals.[12] Other mathematical approaches involve a rule-based approach, in which 'rules' (that represent molecular systems such as those that connect a receptor to the changing transcription of a gene to changing cell behaviour) in a computer program are used to interpret simulated physicochemical parameters such as morphogenetic fields and which, over a broad range of parameters, reproduce biologically-plausible branching patterns.[13]

As mathematical study of branching has grown, so has study at the cell-biological level. Light-microscopic studies of the Victorian era indicated the basic arrangements of cells in tissues undergoing branching and identified key cellular components such as neuronal growth cones, which can control branching at a single-cell level.[14] In the last century, ultrastructural studies, made possible by the electron microscope, indicated that cells driving branching could show various specialisations such as altered extracellular matrix etc.[15] Over the last fifty years or so, these observations have been joined by biochemical analyses, by culture techniques and by experimental interventions that have allowed specific biochemical constituents to be correlated with particular aspects of morphogenesis.[16] Most recently, genetic manipulation has enabled experimenters to make exact and known changes to the genome and correlate these with both normal development and also with congenital disease.

Historically, most researchers into branching have made their strongest connections with others working on different aspects of their chosen organism or tissue, rather than with those studying branching in other systems. This pattern has begun to change, with the realization that the same families of molecules and the same patterns of cell behaviour seem to turn up in system after system. It is still not clear to what extent mechanisms of branching morphogenesis are truly conserved across organs and organisms,[17] but a number of conferences devoted to aspects of branching have shown the value of experimentalists immersing themselves in the biology of each other's systems and, most particularly, of an improved dialogue between biological data and mathematical modelling.

It is for this reason that a set of people involved in studying many different aspects of branching have come together to produce this book. It is impossible, in a volume of reasonable size, to cover everything and, recognizing this, we have tried to pick the topics in which understanding is growing at its fastest and which seem to relate naturally to each other. As Editor, I have very much enjoyed reading the contributions of all of the other authors of this book; I hope that you do too.

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CHAPTER 2

Branching Morphogenesis in Vertebrate Neurons

Katherine M. Kollins and Roger W. Davenport

Abstract

Within the developing vertebrate nervous system, strict control of branching morphogenesis is essential for establishing appropriate circuitry, since the geometry of neuronal arbors critically influences their functional properties. Thus, identification of the specific molecules and mechanisms involved in regulating neuronal branching morphogenesis has been the focus of intense study within recent decades, producing tremendous advances in the understanding of neuronal differentiation. Intrinsic regulation of branching morphogenesis arises through a combination of background gene expression, structural constraints imposed by cellular dimensions, biophysical properties of intracellular cytoskeletal elements, and cell-autonomous control of arbor topology and branching probability. Epigenetic influences on the pattern of branching morphogenesis instead arise from temporally or spatially constrained microenvironmental cues including homotypic and heterotypic cell-cell interactions, substrate-bound and diffusible chemoattractants and chemorepellents, hormones and growth factors, and patterns and levels of electrical activity. Ultimately, such signaling must converge at the level of the cytoskeleton, with the structural changes characteristic of neuronal branching arising through dynamic regulation of the actin cytomatrix, microtubules, and a variety of microtubule-associated proteins. This review provides a comprehensive summary of the current understanding of branching morphogenesis in developing vertebrate neurons, emphasizing recent findings describing key cellular mechanisms and molecular signaling pathways underlying branch formation and stabilization.

Introduction to Branching Morphogenesis in Vertebrate Neurons

Since the pioneering neuroanatomical studies of Santiago Ramón y Cajal beginning in the late 1800s, morphology has emerged as one of the main criteria used for identifying and characterizing distinct populations of neurons.[1-13] As the vertebrate nervous system develops, individual neurons undergo significant morphological changes through a sequence of neurite outgrowth, arborization, and synaptogenesis, leading to the maturation of an astoundingly diverse array of phenotypes (Fig. 1).[3,6-8,14-19] Due to the generation of particular structural attributes through this sequence of morphogenesis, critical functional properties begin to emerge, allowing the establishment of appropriate activity patterns within the developing neuronal network.[8,14,18,20-33] For example, since the majority of CNS synapses are localized to neuronal arbors, changes in arbor surface area influence the reception, integration, and transmission of electrical activity.[2,8,24,25,34-38] Consequently, dynamic regulation of neuronal branching morphogenesis is especially critical for both generating and maintaining the functional organization of the nervous system. While many of the mechanisms underlying branching morphogenesis are likely to be conserved among neuronal populations, the tremendous diversity in neuronal

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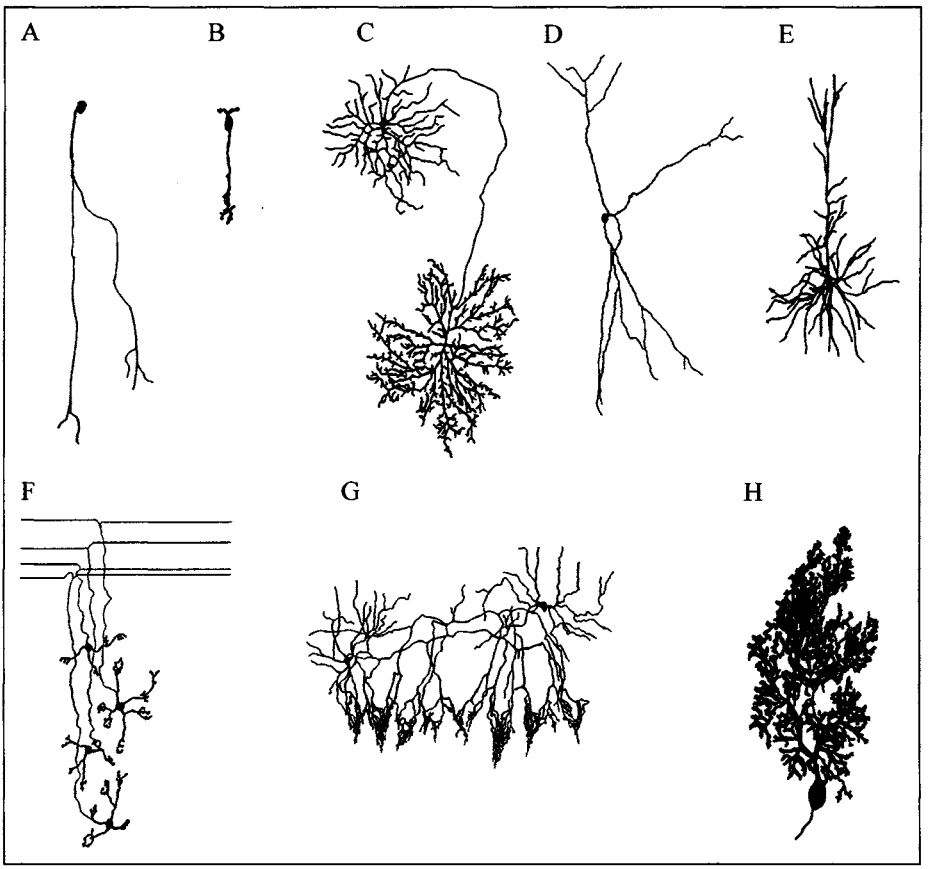

Figure 1. Variations in vertebrate neuronal morphology. A) Sensory neurons of the dorsal root ganglion, categorized as pseudo-unipolar neurons, produce a single elongated and fused axonal process that bifurcates into two functionally distinct branches, but no dendrites. In contrast, B) retinal and olfactory bipolar neurons develop both a single axonal process and a single arborizing dendrite. C) Visual system amacrine and horizontal cells lack typical axons, although specialized presynaptic and postsynaptic regions of dendritic processes exist. D) Neurons of the lateral geniculate nucleus, providing the link between retinal input and the primary visual cortex, are characterized by robust axonal arborization, but limited dendritic elaboration. Conversely, E) hippocampal pyramidal neurons possess distinct functional subpopulations of apical and basal dendrites, and an elongated axon which gives rise to multiple collateral branches. Within the cerebellum, F) granule neurons develop a signature "T-shaped" axon and several unbranched dendrites ending in claw-like termini. A significantly elaborated axonal structure is achieved instead by basket cells (G), which produce moderately arborized dendrites but numerous axon collaterals that form basket-like cages around Purkinje cell somata. In contrast, (H) Purkinje cells develop a planar highly-arborized dendritic tree studded with actin-enriched dendritic spines, but a relatively simple axon. Figure constructed with permission from data presented in references 1, 3, 14, 17, 40, 79, 440, 454 and 635.

cytoarchitecture suggests that some cell-type-specific differences in the regulation of arborization also must arise.[3,6-8,14-19,21-33] Through decades of intensive research, significant progress has been made toward identifying and characterizing the numerous extracellular molecules and cell-cell interactions that regulate aspects of branching morphogenesis. However, the subcellular molecular regulation of branching remains poorly understood. In fact, the degree to which variation in branching architecture among neuronal populations reflects fundamental

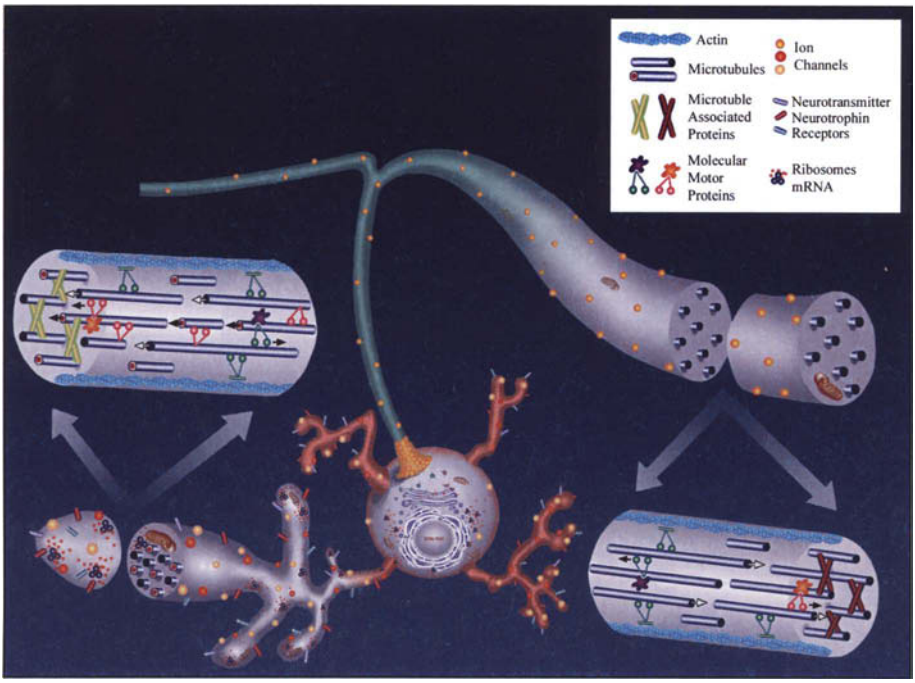

Figure 2. Schematic illustration of the distribution of cellular constituents in a well-polarized neuron. Neuronal morphogenesis typically culminates in the extension of an elongated primary axon and multiple short primary dendrites. Mechanisms that produce local subcellular differences in molecular and cytoskeletal constituents are necessary both for generating neuronal polarity and for triggering branching morphogenesis. At the ultrastructural level, the dendritic domain contains Golgi elements, rough endoplasmic reticulum, mRNA, tRNA, polyribosomes, transcription initiation factors, and a mixed population of plus-end and minus-end distal oriented microtubules. In contrast, the axonal domain excludes biosynthetic machinery but retains specific synapse-related proteins including Na^+ and K^+ channel isoforms, and a characteristic uniform population of plus-end distal oriented microtubules. Fully differentiated neurons thus display an asymmetrical distribution of cytoskeletal elements, cytoskeletal-associated stabilizing proteins, molecular motor proteins, organelles and vesicles, cytoplasmic and cell-surface proteins, and plasma-membrane components. Figure reprinted with permission from K.M. Kollins, constructed from data presented in references 8, 9, 36, 39, 40, 44, 47, 49 and 53.

differences in the control of arborization, or instead represents cell context-dependent modulation of common regulatory pathways, remains largely unclear. Therefore, one significant aim of current research is to identify regulatory pathways that underlie branching morphogenesis for all neuronal populations, and the degree to which branching may be modified through cell context-specific cues.[2,8,26-29,38,49,50,54,85-87,156-163]

The Importance of Neuronal Polarity during Branching Morphogenesis

Neurons are specialized secretory cells characterized by the heterogeneous compartmentalization of various cellular constituents into discrete and physiologically significant domains, the axon and dendrite (Fig. 2).[8,39-44] In turn, this asymmetric organization of cellular constituents allows the partitioning of cellular responses, such as electrical impulse reception, integration, propagation, and release of signaling molecules. Most significantly, the cellular specializations that support appropriate functional polarity also generate inherently different influences on the process of branching morphogenesis within the axonal and dendritic domains.[8,39-44]