Neuronal and glial cell biology

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Here, we review progress in our understanding of neuronal and glial cell biology during the past ten years, with an emphasis on glial cell fate specification, apoptosis, the cytoskeleton, neuronal polarity, synaptic vesicle recycling and targeting, regulation of the cytoskeleton by extracellular signals, and neuron–glia interactions.

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Abbreviations

BDNF	brain-derived neurotrophic factor
BMP	bone morphogenetic protein
GFP	green fluorescent protein
NGF	nerve growth factor

Introduction

The past decade has seen explosive progress in our understanding of how neurons and glial cells are generated and of the molecular basis of their unique cell biology. These advances have largely been driven by powerful technological developments, particularly recombinant DNA technology, including techniques such as the yeast two-hybrid system, forward genetics with invertebrates, and reverse genetics using the mouse as a model system. In addition, novel tracers and probes with which to study and manipulate intracellular compartments and signalling have greatly improved the spatial and temporal resolution of our understanding.

We have selected here some of the most important, but poorly understood questions that confronted neural cell biologists a decade ago. We first review advances in our understanding of neuron and glial cell fate. Second, we summarize progress in characterizing the unique internal organization of neurons, including the establishment of neuronal polarity and protein sorting, the cytoskeleton, axonal transport motors, and vesicular trafficking involved in synaptic vesicle biogenesis and secretion. Lastly, we review advances in understanding how neural cell types communicate with each other.

How are neurons and glial cells generated?

Over the past decade, it has become possible to identify, purify, and culture neural stem cells. This advance, combined with the identification of the highly conserved proneural transcription factors that help determine neuronal fate, has produced great strides in our understanding of how different neural cell types are specified (see also the review, in this special issue, by Jessell and Sanes, pp 599–611). Recent *in vitro* and *in vivo* studies have provided compelling evidence that stem cells are present both in the developing and in the adult brain (for reviews, see [1,2]). In the developing CNS, radial glial cells appear to have the potential not only to guide newly born neurons, but also to self-renew and to generate both neurons and astrocytes [3]. Radial glial cells are heterogeneous, as revealed, for example, by the expression of Pax6 in radial glial cells of the developing cortex, but not of the basal telencephalon. Radial glial cells are also affected in *Pax6*-deficient mice, which show severe developmental abnormalities in the developing cortex [4]. Isolated stem cells can be directed to express proneural transcription factors and to become neurons in response to a variety of instructive extracellular signals. Only very recently, however, are inroads finally being made in understanding how glial cell fate becomes specified. A variety of newly identified soluble signals, including ciliary neurotrophic factor (CNTF), bone morphogenetic proteins (BMPs), and neuregulin-1, direct multipotential stem cells to become glial cells [5,6]. In addition, the Notch signalling pathway — a contact-mediated signalling pathway long known to neurobiologists for its ability to inhibit stem cell differentiation — has recently been found to be a powerful inducer of glial differentiation, including Schwann cells, retinal Müller cells, and radial glial cells in the cerebral cortex (for a review, see [7]).

How do these signals induce glial cell differentiation? Disappointingly, the molecular basis of invertebrate and vertebrate glial differentiation, so far, turns out to have little in common [8]. However, transcription factors that help specify glial fate are finally starting to be identified. In particular, two homologous basic helix-loop-helix proteins called Olig1 and Olig2 have just been discovered that appear to promote oligodendrocyte differentiation [9,10]. However, these DNA-binding proteins are not sufficient on their own to induce oligodendrocyte development, and no transcription factors have yet been identified that are sufficient to induce astrocyte differentiation. Transcription factors that specifically silence neuronal genes have also been identified [11], but the possible role of these silencers in maintaining the glial cell differentiation state is unknown. Still very little is known about the molecular mechanisms controlling the differentiation of astrocytes, including their considerable diversity — clearly an important area of work for the coming decade.

How do neurons and axons die?

One of the major cell biological advances of the past 10 years has been the elucidation of the biochemical pathways leading to programmed cell death. Forward genetics, using for a

large part *Caenorhabditis elegans* and chromosomal translocation in human, has allowed the delineation of the major components involved in programmed cell death (for reviews, see [12,13]). The developmental death of many neuronal populations seems to use biochemical mechanisms similar to those used by other cells. It is now possible to prevent the death of neurons by overexpressing anti-apoptotic factors such as bcl-2 or by removing pro-apoptotic proteins such as bax. Consequently, more neurons than normal are found as a result of the absence of the cell death normally seen during development. In addition, cell death is also prevented when survival factors such as nerve growth factor (NGF) are eliminated [14]. Thus, it is now possible to study the function of NGF or of other such factors beyond their roles as necessary survival agents. In the absence of NGF, for example, neuronal cell bodies are smaller and devoid of neuropeptides normally present in these cells.

Whereas the death of neuronal cell bodies seems to use mechanisms defined for other cell types, it is still unclear how axons are eliminated. In particular, there is evidence that axons degenerate without the involvement of caspase-3 [15]. The mutation *wallerian degeneration* is interesting in this regard. It seems to be cell autonomous, and the results obtained so far indicate that axons can persist far longer than neuronal cell bodies in this mutant [16]. To which degree the cell death machinery may be compartmentalized in neuronal cell bodies, dendrites and axons is unclear at this point.

How is the cytoskeleton specialized to achieve unique neuronal functions?

As in all cells, there are three major types of cytoskeletal elements in glial cells and neurons: intermediate filaments, microtubules and actin. What functions do each of these subserve in neurons and glia?

Intermediate filaments

Neurofilaments and glial filaments are unique cytoskeletal proteins in neurons and glial cells, respectively. Neurofilament proteins consist of light, medium, and heavy chains, and the targeted disruption of each of the genes encoding these proteins has been accomplished over the past decade. Remarkably, none of the mice knockouts has exhibited overt phenotypes, indicating that the neurofilament genes are not necessary for any of the structural specializations of neurons, such as dendrite or axon growth. However, alterations in axonal diameters and numbers have been reported (for a review, see [17]). Similarly, transgenic mice lacking glial filaments exhibit only subtle abnormalities, and even transgenic mice lacking both glial fibrillary acidic protein (GFAP) and vimentin have only a minor phenotype [18]. Thus, as is also true for many non-neural cell types, the functions of intermediate filaments in neurons and glial cells remain largely mysterious.

As with non-neural tissues, intermediate filaments have been remarkably useful for characterizing specific cell types in the nervous system, and, in this regard, the intermediate filament nestin deserves special mention [19]. For reasons that remain entirely unclear, the expression of nestin is limited to precursor cells in the CNS. Upon differentiation of the precursors to cells of the neuronal or glial lineage, nestin expression is downregulated and is replaced by the expression of cell-type-specific intermediate filaments.

Microtubules and axon transport motors

During the past decade, it has been found that microtubules work in concert with specific transport proteins to deliver proteins and organelles to remote regions of neurons [20,21]. There has been an explosion of new information about the nature of these transport proteins. In brief, a large family (with 18 genes in *C. elegans* and many more in mammals) of kinesin proteins has been identified that binds to certain types of cargoes and move them along microtubules in anterograde fast axonal transport and possibly also slow axonal and dendritic transport. Kinesins are about half or less the size of dyneins, which constitute a smaller family of proteins mediating retrograde axonal transport along microtubules. Only two dynein genes have been identified in *C. elegans*. Membrane-bound organelles and vesicles are transported by kinesin and dynein proteins, whereas more conventional motor proteins transport soluble components such as intermediate filaments. Thus, neurons use various types of microtubule-associated motors to control precisely the direction of transport to either axons or dendrites and to transport distinct organelles and proteins to their final destinations. Important future questions will be to understand the polarity of targeting (see below) and to what extent axonal transport processes mediate various types of signalling. Many questions also remain about the specificity of these motor proteins for their cargoes.

Actin

The actin cytoskeleton has been shown to play an important role in the generation and motility of growth cones, spines and dendrites. Axonal growth cones are highly motile structures that transduce extracellular signals and direct neurite growth (for a discussion of guidance cues, see the review by Jessell and Sanes, in this issue, pp 599–611). Major cytoskeletal rearrangements underlie growth cone guidance and growth cone motility. F-actin assembly at the leading edge of growth cones is regulated by a large variety of recently identified proteins [22]. Axon advance also depends upon microtubules, though the exact interactions between microtubules and actin in the growth cone are still uncertain.

Dendritic spines have long been known to be enriched in actin (for a review, see [23]). Studies utilizing actin tagged with green fluorescent protein (GFP) have shown that spines are highly motile structures. As spines are typically covered with presynaptic terminals, these results suggest that the presynaptic and postsynaptic complex may be constantly moving together, implying that mechanical stability may be necessary for synapses to be maintained.

Recent work by several laboratories has also revealed that new spines are generated during the process of long-term potentiation, indicating that significant shape changes, presumably actin-mediated, take place following strong presynaptic stimuli (for a review, see [24]).

The cytoskeleton also interacts with many different signalling systems. Not only can extracellular signals control its stability (see below), but the cytoskeleton also increasingly appears to play a variety of crucial functions in mediating signal transduction. In particular, microtubules have been found to interact with downstream molecules involved in the hedgehog, Wnt, JNK and ERK pathways [25]. Microtubules thus contribute to signal transduction by at least three different mechanisms, including sequestering and release, delivery, and scaffolding of signalling molecules.

How do neurons become polarized?

Neurons are arguably the most highly polarized cells in our bodies, bearing two molecularly and functionally distinct types of processes, axons and dendrites. How do these two domains initially develop? Much of the work on neuronal polarity relies on the observations made with cultured, embryonic pyramidal neurons (for a review, see [26]). It was found that these neurons initially generate several equivalent processes and that neuronal polarization begins when the cell selects one of these processes to become an axon [27]; the other processes then form dendrites. At this stage, microtubules in axons have their polymerizing (plus) ends pointed distally, but in dendrites they can orient in either direction. While a great deal of progress has been made in understanding how proteins are targeted to dendrites and axons after they are initially formed (see below), it is still unknown how this initial polarization of neurons takes place. Recent studies suggest that it may take place without axonal or dendritic sorting of membrane proteins [28]. Remarkably, it has been found that local actin breakdown (such as that induced by an actin depolymerizing drug) is sufficient to initiate axon formation and induces many axons to form on a neuron. These findings demonstrate that a functional actin cytoskeleton is necessary for neuronal polarization. Local actin breakdown, most likely as a result of an external ligand that may involve activation or inactivation of a Rho-family GTPase (see below), may allow microtubules and organelles to enrich in a neurite, leading to axon formation. In this context, it is interesting to note that the neurotrophin receptor p75, a glycoprotein expressed by many developing neurons, constitutively activates RhoA, and that neurotrophin binding to p75 decreases Rho activation and enhances neurite outgrowth [29]. Microtubule invasion into the axon is then crucial for axon elongation [30]. Once established, neuronal polarization is maintained by specific targeting mechanisms.

How do proteins get targeted to different domains? It was proposed 10 years ago [31] that neurons may share membrane protein sorting mechanisms with polarized epithelial cells — with the axonal cell surface analogous to the apical plasma membrane and the somato-dendritic cell surface analogous to the baso-lateral membrane. Indeed, baso-lateral targeting signals used in other epithelia do target neuronal proteins to dendrites, whereas apical targeting signals do not target neuronal proteins to axons [32,33]. These findings suggest that the entire neuronal surface in the brain is equivalent to the baso-lateral surface and that the neuron has evolved unique sub-partitioning mechanisms of the baso-lateral domain into dendritic and axonal compartments [34]. While neurons share some dendritic targeting signals used for baso-lateral epithelial targeting in other epithelial cells, the universality of baso-lateral targeting signals may not be complete as novel targeting signals have been identified in some neuronal proteins [35]. In contrast, most apical proteins expressed in neurons are targeted to both dendritic and axonal domains [32,33], raising the question of how neuronal proteins can be localized predominantly or exclusively in axons. The answer has recently been obtained by using time-lapse microscopy to visualize the transport of GFP-tagged dendritic and axonal proteins within neurons [36]. Whereas dendritic proteins were preferentially transported directly to dendrites and excluded from axon, axonal proteins were transported to both dendrites and axons. However, axonal proteins accumulated only in axonal membranes, implying the existence of a 'selectivity filter' downstream of transport, presumably reflecting either an inability to fuse with or preferential removal from the dendritic membrane. Finally, once proteins are sorted to their appropriate domains, mechanisms are needed to maintain this distribution. In epithelial cells, this function is served by tight junctions that restrict diffusion of proteins between baso-lateral and apical domains. A similar diffusion barrier exists in the initial segment of axons [33]. After neuronal polarization, dendrites and axons become interconnected by synapses. The epithelial adherens junction may be the evolutionary antecedent of the chemical synapse, as they share identical adhesive elements and serve signalling functions [34].

Although it has long been known that both mRNAs and polyribosomes are found in dendrites, only in the past decade has it become clear that specific mRNAs can be targeted to specific cell regions, such as dendrites and myelin, and that local translation actually takes place [37,38]. Neurons may use similar mechanisms to those used by the *Drosophila melanogaster* egg to regionalize specific mRNAs. For example, cis-acting sequences found in the 3′ untranslated region of mRNA transcripts for CAM kinase II and beta-actin bind to RNA-binding proteins such as Staufen, which is required for their proper localization (for a recent review, see [39]). These mRNAs are specifically transported to synapses on RNA-containing granules moving along microtubules in dendrites. These granules contain the proteins needed for local translation at the synapse. Remarkably, the translocation of at least some mRNAs seems to be dependent on activity. In particular, the mRNA coding for Arg3.1/Arc is rapidly enriched specifically in those dendritic sites corresponding to synapses with

increased activity [40]. It has also recently been found that neuronal activity regulates the local synthesis of proteins at synapses, resulting in enduring modifications in synaptic efficacy [41,42]. The challenge for the future is to understand how synaptic activation leads to translational activation and how these newly synthesized proteins create long-lasting modifications in synaptic function.

How are synaptic vesicles created, secreted and recycled?

Yeast genetics, GFP fusion proteins, perforated cell and cell-free systems, and the fluorescent membrane dye FM1-43 are all powerful new tools developed over the past decade for the investigation of membrane endocytosis and exocytosis [43,44]. In addition, the characterization of the major proteins associated with synaptic vesicles and the action of specific toxins on proteins essential for vesicle fusion have contributed to distinguish this area as the one having experienced the most spectacular progress in neuronal cell biology over the past 10 years. It now appears that synaptic vesicles are not formed in the perikaryon and transported to the nerve terminal; rather, they are formed locally at the synapse [44]. The membrane proteins found in synaptic vesicles are targeted to axons (see below), anterogradely transported down axons in membrane carriers that are larger than, and precursors to, synaptic vesicles, which are thought to mediate constitutive membrane transport from the trans-Golgi network to the plasma membrane. Different synaptic membrane proteins are transported down the axon by different kinesin protein carriers, with incorporation of the synaptic proteins into synaptic vesicles taking place in the nerve terminal.

How do synaptic vesicles get formed at the synapse? There have been two longstanding views [44]. According to one idea called 'kiss and run', the synaptic vesicle forms a transient pore with the presynaptic membrane through which the neurotransmitter is released, and the vesicle recycles simply by closing the pore and disconnecting from the membrane. An alternative concept involves vesicular fusion and subsequent recycling by clathrin-coat-mediated endocytosis. Recent studies indicate that both possibilities are probably correct. It appears that there are two synaptic vesicle recycling pathways: a rapid 'kiss and run' recycling pathway from the active zone and a slower clathrin plus dynamin-mediated budding plus fission type of retrieval in non-active zone regions. Some evidence suggests that the active zone pathway replenishes a readily releasable pool of vesicles that is released in response to relatively mild stimulation and that the non-active zone pathway replenishes a reserve pool of vesicles released only with longer-lasting, stronger stimulation. It appears likely that small secretory proteins such as the neurotrophins may be stored in such vesicles requiring stronger stimuli to be released.

Enormous progress has also been made on the sequence determinants present in synaptic vesicle proteins that are involved in endocytosis and targeting to synaptic vesicles [44], as well as the synaptic proteins mediating membrane fusion itself (for a recent, detailed review on membrane fusion and exocytosis, see [45] and the review by Jan and Stevens, in this issue, pp 625–630). Many questions remain to be answered. One of the somewhat neglected areas of research includes the nature of the specific lipids constituting synaptic vesicles as well as the lipid–protein interactions that mediate synaptic vesicle budding and fusion. Recently, evidence has been obtained for the participation of a lysophosphatidic acid transferase (endophilin-1) in the recycling of synaptic vesicles. In association with dynamin (a protein essential for vesicle retrieval), endophilin-1 is thought to help mediate the invagination of synaptic vesicles by locally altering the lipid composition of the vesicular neck [46].

How do extracellular signals regulate the neuronal cytoskeleton?

During development, neurons must undergo extensive morphological changes in order to pattern themselves appropriately and establish functional connections. Extracellular cues play a pivotal role in these events by activating surface receptors that control signalling pathways that regulate the cytoskeleton [47,48]. These pathways were largely unknown at the start of the past decade. An explosion of new information has revealed that the cytoskeleton is modified by a large variety of cell adhesion molecules, including cadherins, integrins, and immunoglobulin superfamily molecules such as neural cell adhesion molecule (NCAM) and L1 [49,50]. Cadherins are calcium-sensitive adhesion molecules that have an interesting and region-specific pattern of expression in the nervous system. The number of these molecules has increased dramatically over the past few years, and non-classical cadherins have also been discovered [51]. It has also been found that many soluble signals, including neurotransmitters, neurotrophic factors, and guidance molecules such as semaphorins, regulate the actin cytoskeleton, which has emerged as a key mediator between signal transmission and anatomic plasticity at synapses [23], just as it is between guidance signals and axonal growth. The exact intracellular mechanisms by which membrane receptors regulate the actin cytoskeleton at dendritic and axonal growth cones are currently the subject of intensive study, and it appears that small GTPases, including rac, rho and cdc42, mediate many of these signalling events. How these small GTPases are regulated by membrane receptors is only just becoming clear (for a review, see [48]). As discussed above, neurotrophins have been shown to regulate the activity of RhoA through binding to their p75 receptor. As the release of neurotrophin is activity dependent, these observations point to a molecular cascade explaining how active presynaptic terminals may modify the shape of their postsynaptic targets. The activity of membrane receptors such as LAR tyrosine phosphatase, which is thought to be regulated by matrix ligands and whose activity is also regulated by an intracellular non-receptor tyrosine kinase Abl, is coupled via the guanine

exchange factor trio/Dock to Rho and Rac, which in turn regulates the cytoskeleton at growth cones [52,53]. A number of effector proteins that couple the activity of activated small GTPases to the actin cytoskeleton have been identified [47,48]. Importantly, it has also been found that these cytoskeletal events can in turn regulate cell adhesion [49]. Exploiting these new advances for a better understanding of the intracellular mechanisms by which extracellular signals guide growing axons will be a crucially important area of research during the coming decade.

How do neurons and glial cells communicate?

Over the past 10 years, many new signalling receptors and ligands have been identified in the developing and adult nervous system. These signalling ligands regulate survival, proliferation, and differentiation, and include soluble signals such as neurotrophins, cytokines, neuregulins, BMPs and contact-mediated signals, such as Notch ligands, Wnts, and ephrins. Some important themes, common to some of these signalling molecules, have emerged during the past decade from intensive study of one of these signalling families, the neurotrophins. NGF, brain-derived neurotrophic factor (BDNF), and the neurotrophins NT-3 and NT-4/5 are small secreted proteins whose main function 10 years ago was thought to be the promotion of neuronal survival (for a recent review, see [54]). Not only do neurotrophins also kill neurons during development, but it has also become increasingly clear that they are involved in a large number of other events relevant to the function of the nervous system. In particular, the transcription of the neurotrophin genes is regulated (both positively and negatively) by neuronal activity, and the secretion of neurotrophins by neurons is activity dependent (for a review, see [55]). In addition, it is now clear that beyond their influence on neuronal survival, neurotrophins can also modify the shape of dendrites in the central nervous system (for a review, see [56]). Work over the past 10 years has also revealed that neurotrophins are more than trophic factors, as they have been found to subserve a large variety of physiological roles in the adult nervous system. For instance, NGF is involved in many aspects related to the pathophysiology of pain (for a review, see [57]), and BDNF regulates presynaptic neurotransmitter release and postsynaptic glutamate receptor activity. BDNF also potently activates a novel form of sodium channel [58], and is clearly directly involved in long-term potentiation in the CA1 area of the rodent hippocampus (for reviews, see [54,56]). Because BDNF can be anterogradely transported, is secreted in response to activity, and has postsynaptic receptors that it can rapidly activate, it has a number of properties in common with classical, small molecular weight neurotransmitters. These findings clearly indicate that neurotrophins play a large variety of different roles in regulating the function of the adult brain, and provide a hint of the large amount of work left to be done in investigating potential similar roles for the many other currently known peptide signalling factors thought currently to play roles only in the developing brain.

The past decade has also seen enormous progress in our understanding of the molecular basis of axon–glial cell interactions. Two areas have been of particular interest. First, how is the generation of myelinating cells and myelination controlled? It has long been proposed that axonal signals control these events, but their nature has not been known. Axonal neuregulin has been found to control the proliferation and particularly the survival of oligodendrocytes and Schwann cells, ensuring a good match between the axon surface area requiring myelination and the number of surviving myelinating cells [59]. Notch signalling has been proposed to be an on/off switch that controls the timing and localization of myelination [60]. Before myelination, axons express Notch ligands, which inhibit oligodendrocyte differentiation and myelination and which are downregulated at the onset of myelination.

Another longstanding goal has been to understand the mechanisms that define nodal and internodal domains along axons. During myelination, voltage-dependent sodium channels become clustered at nodes of Ranvier, and voltage-dependent potassium channels become clustered in the juxta-paranodal regions [61–63]. The nodal axolemma contains a high density of the Na(v)1.6 sodium channel [64] linked to the axonal cytoskeleton by ankyrin-G, which binds to the beta subunits of the sodium channel. The paranodal myelin loops are anchored to the axon by the axonal contactin-associated protein Caspr, a vertebrate homologue of neurexin IV, also called paranodin; its myelin binding partner is unknown but has been speculated to be the myelin-specific lipid galactocerebroside. The juxta-paranodal axolemma contains the potassium channels Kv1.1 and Kv1.2 and their associated β2 subunit, which all are tightly associated with another axonal protein Caspr2, a PDZ-binding protein and Caspr homologue. It has been found that myelinating cells (i.e. oligodendrocytes and Schwann cells) secrete signals that are essential for sodium channel clustering, as well as for clustering of the juxta-paranodal potassium channels. An important goal for the next few years will be to identify these glial-cluster-inducing signals. Other goals will be to understand the mechanism of the myelination process itself, and the mechanism by which myelinating cells induce the axon to increase in radial diameter [65].

A large area of continued ignorance remains in that we know little about the normal functions of astrocytes, a major cell class in the brain. Long regarded as passive support cells, the past decade has produced much evidence that astrocytes may also have a number of important, active roles in the formation and functioning of the nervous system. Certain glia, ependymal cells and sub-ventricular astrocytes turn out to be neural stem cells in the adult brain [1]. Astrocytes respond to neuronal activity with an elevation of their intracellular calcium, which triggers the release of chemical transmitters that can, in turn, influence neuronal activity [66]. Even in the absence of such calcium signals, astrocytes enhance enormously the number of

functional synapses that form between neurons (see [67] for a review). Amazingly, it has even been discovered that astrocytes can directly couple to neurons *in vivo* and directly regulate neuronal activity [68]. Thus, the potential active roles of glia in CNS formation, function, and plasticity should prove to be an exciting area of research during the coming decade.

What can we expect during the coming decade?

Just as new methodology has driven the advances of the past decade, technological advances will most likely drive future progress. In general, the past decade has elucidated many new molecular components of cells, and provided a framework for basic cell biological processes. But there is a long way to go before we understand how the individual components cooperate to give rise to specific functions. Our understanding of neuron–glial cell interactions and glial cells specializations such as myelination, in particular, remain in their infancy, as many glial functions appear to be unique vertebrate adaptations that have so far not been amenable to systematic genetic approaches. Likewise, some important signalling systems such as the neurotrophins seem to be absent from the most widely used model organisms such as *C. elegans* and *Drosophila melanogaster*. It is to be expected that forward genetics using vertebrates, including the mouse in particular, may allow a novel degree of complexity to be approached in the next decade.

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