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A Gathering of Neuronal Cytoskeletal Scientists in South America

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This essay attempts to capture the spirit of our recent meeting in Chile entitled "Emerging Concepts in Neuronal Cytoskeleton." The purpose of the meeting was to bring together scientists from South American countries with colleagues from the USA and the global community, to inspire and motivate the next generation of scientists from South America who share a fascination of the internal architecture of the neuron.

Key Words: neuron, axon, dendrite, cytoskeleton, neuronal, polarity, axonal transport, microtubule, neurofilament, actin

Introduction

In April 2011, over 50 scientists and students met in Chile to present our most recent findings and to discuss our current understanding of the neuronal cytoskeleton. The auspicious occasion took place in a beautiful venue in Santa Cruz, nestled in the wine country. The main purpose of the meeting was to enlighten and inspire graduate students from South American countries, by bringing together scientists from their own countries with colleagues from the USA and other countries around the globe. In so doing, we had the opportunity for vibrant interactions and discussions that inspired us all toward new ways of thinking, potential collaborative interactions, and new avenues for our research. It is fitting that this meeting focused on neuronal cytoskeleton, in that some of the most important and seminal advances in this field have been made in laboratories in South America or by scientists from South American countries working elsewhere in the world. The meeting was entitled "Emerging Concepts in Neuronal Cytoskeleton," and was Co-chaired by the authors of this meeting synopsis. Highlights of the meeting included a stirring Plenary Lecture by Alfredo Caceres (Instituto Investigación Médica Mercedes y Martín Ferreyra), oral presentations from over 20 internationally recognized scientists, a number of outstanding posters from graduate students, and a highly participatory business meeting to discuss future gatherings of this kind.

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Neuronal Cytoskeleton in South America

Cellular and molecular neurobiology in South America is historically linked to the cytoskeletal field. In particular, scientists from Argentina since the late 1970s have been responsible for many breakthroughs, such as the pioneering work of Hector S. Barra on the tubulin tyrosination cycle. Barra described how tubulin can cyclically incorporate/release a C-terminal tyrosine residue at the alpha subunit. He also noted that the content of detyrosinated tubulin within a brain microtubule correlates with its stability. Later in the 1980s, Alfredo Caceres and Carlos Dotti (a onetime mentor and graduate student in Argentina) began a long series of influential publications. Caceres (working with Lester Binder, Ossie Steward, and Gary Banker) reported that a microtubule-associated protein (MAP), termed MAP2, was found specifically enriched in the dendrites of cultured hippocampal neurons, suggesting that some MAPs are specifically targeted to the axonal or somatodendritic compartment of the neuron. By that time, the neuronal polarity field had become active with the establishment of primary neuronal cell culture of rat hippocampal neurons by Banker's group. Dotti, working in Banker's laboratory, described the sequential

steps of hippocampal neuronal differentiation in a seminal paper, proposing the discrete and stereotyped stages that define the acquisition of neuronal polarity in vitro. By the 1990s, both Caceres and Dotti had further demonstrated the importance of various cytoskeletal proteins in neuronal differentiation. Caceres used antisense oligonucleotides to study the importance of MAPs in axonal and dendritic differentiation. Dotti's group demonstrated that local actin dynamics are finely controlled to elicit axonal elongation. With the refinement of genetically modified models in the 2000s, other cytoskeleton proteins were shown to be important for neuronal differentiation. This is the case for MAP1B, which was shown by the Chilean scientist Christian Gonzalez-Billault to be an important player in axonal elongation. Currently, there are many more South American scientists working in the neuronal cytoskeletal field, many of whom participated in the Workshop, and contributed to this special issue of *Cytoskeleton.* With the mentorship of outstanding South American researchers already in place, we envision that continued Workshops and International Collaborations will spur a vigorous progeny of young South American scientists as new leaders in neuronal cytoskeletal research.

Starting With Microtubules

Upon arrival at the venue midday on Sunday, the participants spent the remainder of the day focused on microtubules. In keeping with the "emerging concepts" theme of the meeting, three of the oral presentations revolved around microtubule-severing proteins, a relatively new addition to the robust literature on neuronal microtubules. To date, most studies on how microtubules become organized in neurons have focused either on their dynamic properties or on their transport by molecular motor proteins. Microtubule severing is a physiological process that is relevant to both, in that breaking a single long microtubule into many shorter ones has broad functional consequences. The severing event creates a higher number of microtubule free ends that are available to undergo either assembly or disassembly. In addition, the bona fide transport of microtubules in neurons appears to be limited to rather short ones, and hence severing may enhance mobility within the microtubule array.

Microtubule-severing proteins are enzymes that act in the form of hexamers that use the energy of ATP hydrolysis to break the lattice of the microtubule. To date, the best studied of the severing proteins are katanin and spastin. Antonina Roll-Mecak (National Institutes of Health) began the session by discussing her seminal work on the biochemical mechanism by which these proteins actually break the microtubule lattice, specifically by yanking on a tubulin subunit within the lattice. Until this high-resolution structural work was done, it was a mystery how the spastin or katanin hexamer, too small to fit around the microtubule and yet unlikely to be able to get inside the lumen of the microtubule, caused the microtubule to break. Roll-Mecak's work elegantly demonstrates that the hexamer sits on the surface of the microtubule where it generates the yanking effect, rather than wrapping around the outside or the inside of the microtubule. Jonathan Wood (University of Sheffield) then spoke on how mutations to spastin might impact the microtubule array of the axon in such a way as to cause the degeneration of nerves observed in Hereditary Spastic Paraplegia. His studies, using live-cell imaging on Zebra fish embryos, suggest that diminution in microtubule-severing activity can have notable impact on axonal vitality. In the last presentation on microtubule severing, Peter Baas (Drexel University) presented a novel hypothesis as to how dysregulation of microtubule severing may contribute to another class of neurodegenerative diseases, namely tauopathies such as Alzheimer's. The hypothesis is that loss of microtubules, believed to contribute to nerve degeneration in these diseases, may result from too much microtubule severing. This idea is based on evidence that association of tau with microtubules normally regulates the access of katanin to the microtubule lattice. Collectively, these three presentations on microtubule-severing proteins provided a compelling picture of how normal microtubule severing is important for the vitality of the axon and how flaws in the regulation of the severing proteins can contribute to neurodegeneration.

The final presentation of the microtubule session continued on the theme of microtubule loss in damaged axons but focused on injury rather than disease. John Bixby (University of Miami) explained how drugs that preserve microtubules can provide a notable boost to the regenerative response of damaged adult axons. These drugs include taxol, the classic microtubule stabilizer used in cancer therapy, but also novel drugs from an extensive survey that proved to have potent effects on microtubules in the axon as well as on the regenerative response after injury. In related presentations later in the meeting, Carlos Arce (Universidad Nacional de Córdoba) and Yuyu Song (University of Illinois at Chicago) discussed post-translational modifications of tubulin, and how such modifications can contribute to the functional properties of microtubules in the nervous system. Arce's presentation dealt mainly with acetylation of microtubules, while Song's presentation dealt with a newly described modification called polyamination. Given that axonal microtubules are typically more stable than most interphase microtubule arrays, these presentations were interesting in terms of the normal development and maintenance of the nervous system but also in terms of Bixby's data indicating that microtubule-stabilizing drugs could assist damaged neurons to regenerate better.

The microtubule presentations fostered a great deal of discussion, both at the session and at informal gatherings thereafter. One of the major points of interest was how

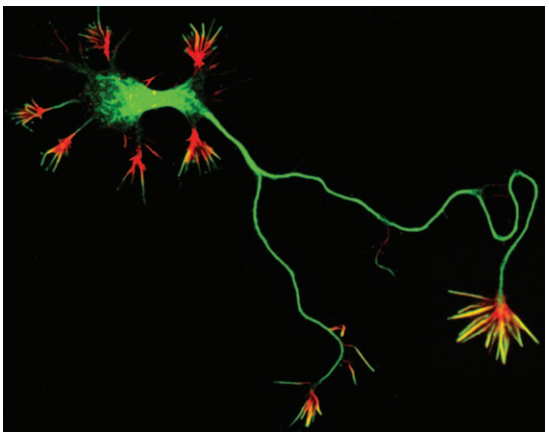

Fig. 1. A confocal fluorescence merged image showing the morphology of a stage 3 hippocampal pyramidal neuron after 1 day in culture. The cell was labeled for the RhoA guanosine nucleotide exchange factor Lfc (green) and F-actin (red, Phalloidin). Lfc associates with microtubules within the axon and minor neurites, and also localizes at the Golgi apparatus and filopodial extensions at the periphery of growth cones. The image was taken using an Olympus FluoView 1000 spectral confocal microscope. Image provided by Cecilia Conde and Alfredo Caceres.

the amazing advance of basic science, exemplified so dramatically in Roll-Mecak's presentation, has enabled the microtubule community to make significant inroads not only toward understanding disease and injury of the nervous system, but also in developing potentially powerful therapies. The Wood presentation gave rise to discussion as to whether Hereditary Spastic Paraplegia is caused by insufficient microtubule severing or by toxic properties of the mutant spastin. The Baas and Bixby presentations gave rise to discussion on exactly which strategies would be most effective at fortifying the axon against microtubule loss during disease and injury. While low doses of taxol have provided a compelling proof-of-principle demonstration that preserving and perhaps stabilizing microtubules can foster axonal regeneration, it was brought up that taxol is infamous for producing neuropathy when used to treat cancer patients. It was also brought up that stabilization of microtubules may not be the most effective means of preserving microtubules from excess severing, and that other strategies may be necessary. Use of newer approaches such as experimentally altering microtubule acetylation, screening for microtubule-active drugs, and the use of a microtubule-active neuroprotective peptide were discussed.

The plenary lecture by Caceres introduced many of us to an exciting new category of microtubule-regulatory proteins called GEFs (microtubule-associated guanosine nucleotide exchange factors) and also provided a personal perspective on the history of the neuronal polarity field. The lecture focused on how GEFs (namely Tiam1 and Lfc, and its inhibitor Tctex-1) regulate Rho-GTPase activity during the formation of the axon. Figure 1 shows an example image from the presentation by Caceres. This work accentuated just how complex neuronal polarity is, with a variety of factors and pathways involved. Caceres inspired a great deal of discussion on exactly what neuronal polarity is, which proved to be a persistent theme of interest throughout the meeting. This was a wonderful close to the first day and an ideal enticement for the morning session of the second day, which was devoted to contemporary advances in neuronal polarity.

Neuronal Polarity

The second day opened with a session on neuronal polarity, a very appropriate topic given the essential role of cytoskeletal elements in establishing neurons as the single cell type in all of nature whose polarity is most intimately related to its unique functions. Neurons would be highly polarized if they only extended an axon, but they become even more polarized by developing a second type of process, the dendrite. A typical vertebrate neuron extends a single axon, which is a long slender process that is effectively unlimited in its growth potential. The axon is specialized to transmit information over potentially long distances. By contrast, neurons extend multiple dendrites, which tend to be short, stout tapering processes, highly specialized to receive and process incoming information. In addition to their functional and morphological differences, axons and dendrites also differ compositionally, with regard to organelle distribution, membrane-associated receptors, and content of proteins such as MAP2, mentioned earlier. Understanding the cell biology of the neuron is inextricable from understanding how its polarity is established and maintained. In attendance at the workshop were several of the most influential scientists who helped shape the early years of research on neuronal polarity, as well as bright new scientists and students driving the field forward. Figure 1, provided by Caceres, shows an example image of a neuron newly polarized, so that the axon has formed (and branched), but the dendrites have not yet differentiated from the remaining immature processes. Figure 2, also provided by Caceres, shows a beautiful image of growth cones of dorsal root ganglion (DRG) neurons, fluorescently stained to reveal actin and microtubules.

In the first presentation of the session, Christian Gonzalez-Billault (Universidad de Chile) discussed how a second messenger triggers the appearance of an axon. He showed that a rise of cAMP is responsible for the activation of two complementary signaling pathways. Specifically, he showed that the exchange protein directly activated by cAMP (EPAC), is a guanine-exchange factor that promotes the activation of Rap1b in a cAMP-dependent manner. He then showed that changes in the activity of EPAC proteins may alter the development of neuronal polarity, thereby providing alternative and complementary mechanisms for the activation of PKA, which has recently been proposed to be essential for the development of an axon. The second presentation of the session was delivered

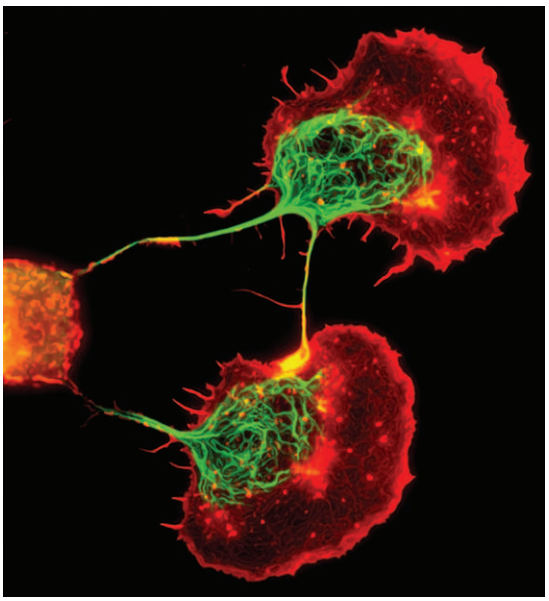

Fig. 2. A fluorescence image showing the morphology of axonal growth cones from a DRG neuron cultured for 12 hours. The culture was stained with a mAb against tyrosinated α-tubulin (green) and phalloidin (F-actin, red). Note that the neuron has extended two short neurites tipped by two prominent growth cones. The central growth cone domain is enriched in tyrosinated microtubules, while the peripheral domain contains a large lamellipodial veil. The image was taken using a conventional inverted fluorescence microscope (Carl Zeiss) equipped with a Hamamatsu CCD (Orca 10) camera. The image was deconvoluted using Metamorph software and further processed with Adobe Photoshop (diffuse anisotropic filtering). Image provided by Jose Wojnacki and Alfredo Caceres.

by Kozo Kaibuchi (Nagoya University). Kaibuchi provided insight into how neurotrophins released in the brain could trigger cellular and molecular changes leading to the establishment of neuronal polarity. He explained that locally released NT-3 is responsible for rapid local changes in calcium levels at the growth cones of elongating neurites. These changes in turn activate calcium-calmodulin dependent protein kinase kinase (CaMKK), suggesting an exciting new mechanism for the involvement of calcium in axonal elongation. In the final presentation of the session, Carlos Dotti (CBMSO and KU Leuven), described the acquisition of polarity as an early event, related to the last cell division of neuronal precursors. Dotti argued, using a *Drosophila* model, that determination of cell polarity in vivo is independent of centrioles. He demonstrated that in the context of centriole deletion, pericentriolar proteins could serve as the initial source of an asymmetric cytoplasmic environment, suggesting that the acquisition of neuronal polarity may be traced back to the initial stages of development. During a stimulating question and answer session, Dotti was challenged to consider the notion that his various findings might be explicable on the basis of microtubule organization, an idea to which Dotti responded favorably.

These were not the only presentations dealing with neuronal polarity, as the topic came up at many of the other oral presentations, as well as the poster sessions. It was also a favored topic during mealtime discussions and social excursions. One interesting discussion was on the very meaning of neuronal polarity, as some of the group felt that too often recent studies have confused axonal growth with neuronal polarity. Axonal specification is a matter of neuronal polarity, it was argued, but the growth of the axon is not. Other participants argued that axonal navigation is surely an illustration of neuronal polarity, since growth cones need to turn in a polarized fashion toward or away from guidance cues.

Molecular Motors and Transport

No workshop or meeting involving neuronal cytoskeleton would be complete without a robust component on axonal transport and the molecular motor proteins that fuel it. Cytoplasmic dynein, as well as members of the kinesin and myosin families of motors, are critically important across cell types in enabling proteins and organelles to actively and directionally move from one location to another. The axon, with its effectively unlimited growth potential, is potentially the greatest challenge in all of nature, for such transport mechanisms. The fact that neurons develop dendrites as well as an axon offers another challenge to the manner by which molecular motors transport specific cargo, as many of the cargoes that are transported into each type of process are not transported into the other type of process.

The session began with a presentation by Gerardo Morfini (University of Illinois at Chicago) that involved the identification of a novel pathway by which cytoplasmic dynein-dependent retrograde axonal transport is activated. In particular, Morfini demonstrated that retrograde axonal transport of neurotrophins involves phosphorylation of specific cytoplasmic dynein subunits. Moreover, he discussed specific protein kinases mediating these phosphorylation events, and the implications of these findings on the pathogenesis of neurodegenerative diseases, including Parkinson's disease. Another aspect of the dynein story was then communicated by Ching-Hwa Sung (Cornell University), who presented fascinating findings indicating that a component of the dynein machinery called Tctex-1 can be dissociated from the dynein complex via phosphorylation, and when this happens, Tctex-1 has important nonmotor function in regulating actin dynamics. Her work shows that phospho-Tctex-1 is functionally involved in both neurite outgrowth of hippocampal neurons and primary cilium resorption of neural progenitors during brain development.

The next presentation, by Don Arnold (University of Southern California), reminded us of how intricately the axonal transport and neuronal polarity topics are intertwined. A long-standing issue in neuronal polarity is how different vesicle-bound membrane proteins are targeted

specifically to dendrites or axons. Most work to date has focused on microtubule-associated motor proteins, but Arnold's work points to potentially powerful roles for the myosins. In particular, he reported on a role for Myosin VI in the localization of axonal proteins, which complements earlier work from his laboratory on targeting of dendritic proteins by myosins. The myosin theme was featured as well in a presentation on mitochondrial transport in the axon by Peter Hollenbeck (Purdue University). Building on his long-standing interests in mitochondria, Hollenbeck showed that depletion of myosins V and VI increase the persistence of mitochondrial movements, suggesting that these two myosins may support the pausing or docking of mitochondria. In the second half of his presentation, Hollenbeck focused on a *Drosophila* model for neurodegeneration in which he showed evidence for suppression of retrograde mitochondrial transport. This work provided an enticement to the session on Tuesday on the cytoskeleton and nerve degeneration.

One of the most interesting discussion points during the session was related to the fact that microtubules have mixed polarity at proximal dendrites, while they are nearly uniformly oriented in the axon. This idea inspired some discussion of a potentially comparable mechanism contributing to organization of actin filaments within the initial segment of the axon, which serves as a diffusion barrier. It was discussed whether these actin filaments defining the axon initial segment have uniform or mixed polarity, possibly contributing to the targeting of molecular components associated with specific myosin motors.

Actin and Neurofilaments

With such a strong emphasis on microtubules thus far in the meeting, it was appropriate that the sessions on Tuesday began with presentations on actin filaments and neurofilaments. Paul Letourneau (University of Minnesota) reported on actin filaments, while Atsuko Uchida (Ohio State University) reported on neurofilaments. Letourneau's presentation also brought to the forefront of the discussions the structure at the tip of the growing axon, the growth cone, which is arguably the region of the neuron that is the busiest with cytoskeletal interactions. He devoted much of his attention to ERM proteins (ezrin, radixin, moesin), which are highly conserved proteins that crosslink actin filaments to transmembrane proteins. In addition, Letourneau reminded the audience of how important cleverly designed experiments have been to our current understanding of the growth cone. In particular, he showed an amazing experiment in which he combined recombinant cofilin with a peptide (Chariot^{TM}) that complexes molecules so that they can cross through the cell membrane. Growth cones were shown to turn toward a gradient of Chariot-complexed cofilin. This experiment is shown schematically in Fig. 3.

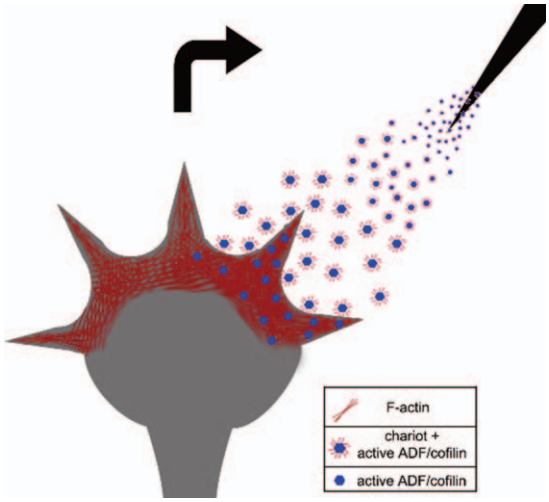

Fig. 3. Schematic illustration of attractive growth cone turning toward a gradient of cell-permeable ADF/cofilin. Figure provided by Bonnie Marsick and Paul Letourneau.

The presentation by Uchida dealt with a novel mechanism of neurofilament behavior in the axon. She presented data suggesting that neurofilaments may be severed in a physiological manner, as has long been known to be the case for actin filaments and more recently discovered to be the case for microtubules (see earlier). More amazingly, she directly demonstrated that pre-existing neurofilaments can join together in an end-to-end fashion in living neurons, which neither actin filaments nor microtubules are known to be able to do. These observations indicate that neurofilament length is regulated by a dynamic cycle of breakage and annealing. If the length of a neurofilament is a crucial determinant of its transport properties (as is the case with microtubules), these observations would have strong implications for the mechanisms regulating neurofilament transport in the axon. As with the Letourneau presentation, a highlight of Uchida's presentation was the technical innovation, in this case the combined use of photoactivatable GFP and mCherry to visualize neurofilament annealing in living neurons.

Among the questions inspired by these presentations was whether the observations made by Uchida might help explain previous reports by other authors in which intermediate filament proteins were shown to form clusters that displayed motility, especially in cells that had been vigorously triturated shortly before observation. Uchida speculated that perhaps neurofilaments that break up during injury might reconstitute themselves through the movement and annealing of short pieces into longer neurofilaments.

Cytoskeleton and Neurodegeneration

The promise of basic research to strongly impact progress on diagnosing and treating human disease is being fulfilled in the arena of neuronal cytoskeleton. Today it is

known that many of the proteins and mechanisms that many of us studied over the years in our basic research programs reside at the heart of neurodegenerative diseases. Such examples include spastin, discussed on the first day of the meeting as a protein whose mutations are the chief cause of Hereditary Spastic Paraplegia. Perhaps the best known example is the protein tau, whose disrupted normal function culminates in a family of diseases called tauopathies, the most prominent of which is Alzheimer's disease. The session on neurodegeneration was a particularly important element to our meeting, given the societal importance of treating diseases. The importance of both basic research, as well as research aimed directly at the diseases that plague human patients, was stressed to the students in attendance.

James Bamburg (Colorado State University) began the session by presenting a novel idea for how actin-based mechanisms contribute to loss of synapses in Alzheimer's disease. Cofilin is activated by dephosphorylation, and this effect can be abnormally heightened by initiators of neuronal dysfunction such as oxidative stress, excitotoxic glutamate, ischemia, and also soluble amyloid beta. As a result, actin is remodeled into elongated cofilin-saturated actin bundles termed "rods." Evidence was presented that these rods enhance or mediate virtually all of the early pathological symptoms of Alzheimer's disease. Bamburg's presentation served as a perfect illustration that stress can convert normal proteins without mutations to pathogenic forms that can contribute to diseases in significant ways.

Two other presentations in the session dealt with tau, which was discussed in the earlier microtubule session with regard to the impact of its loss of function in diseases such as Alzheimer's. Here, Scott Brady (University of Illinois at Chicago) explained why a potentially even more immediate threat to the axon is the filamentous forms that the hyperphosphorylated tau can adopt. He showed that anterograde, conventional kinesin-dependent fast axonal transport (FAT) is reduced by pathogenic aggregated forms of tau. Significantly, this effect involved a mechanism independent of tau's binding to microtubules. Moreover, it is a specific region of tau (amino acids 2–18) that is responsible for this effect. These results argue that pathogenic forms of tau protein activate a toxic gain-of-function mechanism that negatively impacts axonal transport by activating a signaling pathway involving phosphatase PP1 and GSK3β. The result is phosphorylation of kinesin light chains and inhibition of anterograde FAT, as well as phosphorylation of tau. Fitting in very well with Brady's story was a presentation by Patricia Cardona-Gomez (University of Antioquia) on the mechanisms underlying tau's hyperphosphorylation. She presented data indicating a possible interdependency of CDK5 and Rho GTPases on the regulation of tau's phosphorylation, which could go awry in a manner triggering tauopathy.

Cytoskeleton and Dendrites

The evening session on Tuesday focused on cytoskeleton and dendrites. The session included three regular invited presentations and two short presentations selected from the submitted abstracts. In the first presentation, Peter Penzes (Northwestern University) reviewed how actin cytoskeleton regulation and specifically the dynamic behavior of dendritic spines are essential to support neurotransmission. Signaling on mature neurons was analyzed in the context of the guanine nucleotide exchange factor, kalirin, which is an important effector downstream of monomeric GTPases controlling actin dynamics. The role of kalirin in dendrite function was chosen as an example of the mechanisms linking neurotransmission to the dendritic cytoskeleton. In the same context, Tomoaki Shirao (Gunma University) presented his work on the actin binding protein, drebrin. Using FRAP experiments, Shirao demonstrated that the localization and specific accumulation of a drebrin isoform are important to transduce AMPA receptor signaling in dendrites. The participation of extracellular molecules that regulate the function and dynamics of dendritic spines was then discussed in a presentation by Nibaldo Inestrosa (Pontificia Universidad Catolica de Chile), using as a model system long-term cultures of hippocampal neurons. Wnt signaling may be specifically linked to the control of synapses at either the presynaptic or postsynaptic compartments. In his presentation, Inestrosa showed evidence that Wnt-3a promotes presynaptic differentiation, while Wnt-5a is important for postsynaptic development. Interestingly, Wnt-5a effects are mediated through pathways involving CaMKII and JNK.

In the first short presentation, Carolina Montenegro (Universidad de Chile) introduced a new role for MAP1b in adult brain. The absence of MAP1b leads to abnormal development of dendritic spines, most likely due to decreased Rac1 function. The second short presentation, delivered by Maria Paz Marzolo (Pontificia Universidad Catolica de Chile), dealt with the control of receptor trafficking in mature neurons. ApoER2 had been shown to participate in the control of synapses, and in this work, the trafficking of this receptor was shown to be dependent on the endocytic pathway adaptor protein sorting nexin 17.

Mechanisms of Slow Axonal Transport

On Wednesday morning, the closing session of the meeting focused on the most enduringly controversial topic in the neuronal cytoskeletal field, namely slow axonal transport. FAT is the movement of membranous organelles along microtubules in the axon (and to a lesser extent, along actin filaments), while slow axonal transport is the

movement of the cytoskeletal proteins themselves. Slow axonal transport also includes most of the cytoskeletal-associated proteins, as well as a vast array of proteins that are generally considered to be soluble/cytosolic. Early work in the field prompted the structural hypothesis, wherein microtubules, actin filaments, and neurofilaments were posited to move down the axon as polymers rather than as subunits, with the soluble/cytosolic proteins presumably attached in some way to the actin cytoskeleton. Neurofilaments and most of the microtubules (and their associated proteins) were considered to move in "slow component a" (SCa), while actin filaments (and their associated proteins), as well as the soluble/cytosolic proteins were considered to move slightly faster in "slow component b" (SCb). In both SCa and SCb, proteins are conveyed notably slower than in FAT. With regard to neurofilaments and microtubules, after years of controversy, it now appears to be resolved that both are transported in the form of polymers, as predicted by the structural hypothesis. Through contemporary live-cell imaging, we now know that individual neurofilaments and microtubules are transported bidirectionally in the axon at rates comparable to FAT. However, the transport of individual polymers within the array is asynchronous and infrequent, with most polymers not undergoing any movement at any given time. This explains why the collective arrays appeared to move so slowly in the original transport studies. It has now been over a decade since this groundbreaking imaging work was first reported, with significant progress ensuing on the mechanisms that regulate the movements of both types of filaments. The Wednesday session started with updates on neurofilament transport and microtubule transport, and also included exciting new findings on the mechanism by which the soluble/cytosolic proteins are transported in SCb.

Anthony Brown (Ohio State University), who conducted the original live-cell imaging work on neurofilaments and microtubules, started the session with a presentation on his recent progress on neurofilament transport. Using his newer "pulse-escape" method for visualizing neurofilament movements, Brown found that the rate of neurofilament departure from a demarcated zone was more rapid in unmyelinated regions of axons compared to adjacent myelinated regions. These data provide the first direct evidence for a longstanding hypothesis in the field, namely that myelinating cells locally impact axonal morphology by modulating neurofilament transport.

The presentation by Peter Baas (Drexel University) expanded upon his longstanding hypothesis that the distinct microtubule polarity patterns of axons and dendrites (uniform and nonuniform, respectively) are established and maintained by molecular motor proteins that transport microtubules into these neurites with either the plus or the minus end of the microtubule leading. His hypothesis has been that cytoplasmic dynein is the principal

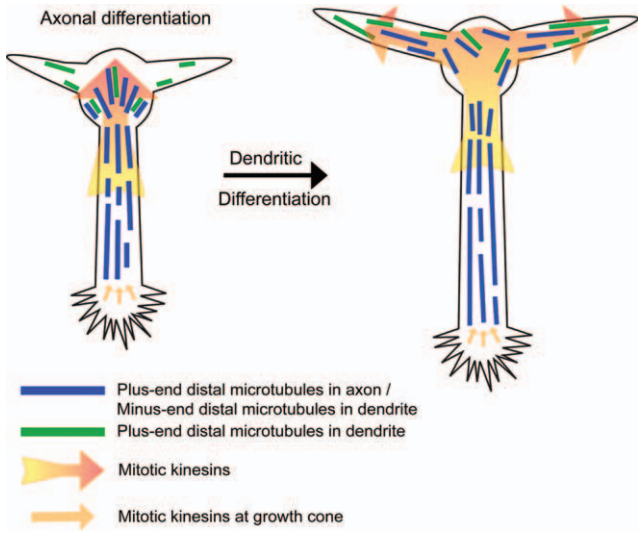

Fig. 4. A model by which mitotic motors coregulate the microtubule polarity patterns of axons and dendrites by attenuating the transport of plus-end-distal microtubules in the axon while promoting the entry of minus-end-distal microtubules into dendrites. Figure provided by Shen Lin and Peter Baas.

workhorse for transporting microtubules with their plus ends leading, and that the remainder of the work is performed by a small number of kinesins usually considered to be mitotic motors. To date, these motors include kinesins 5, 6, and 12. Interestingly, however, the results of recent studies from the Baas laboratory indicate that, in fact, these mitotic motors do not fuel microtubule transport in the axon, but rather they somehow suppress it. A potentially exciting hypothesis is that these motors act, at least in part, at the level of the cell body to restrain the transport of microtubules into the axon, while simultaneously regulating the transport of microtubules of the opposite orientation into the dendrites (Fig. 4). In his presentation, Baas posited that this is the mechanism by which the neuron coregulates the polarity orientation of microtubules in the axon and the dendritic arbor.

Some of the most innovative ideas at the workshop were presented by Subhojit Roy (UCSD), who discussed a new model that he has set forth on the mechanism driving the SCb transport of soluble/cytosolic proteins. To date, SCb has remained the most enduring mystery in the axonal transport field, with virtually no data or even a particularly compelling hypothesis for how presumably soluble proteins could be actively transported. The idea of these proteins simply hitching a ride on moving actin filaments has never been supported by data. Using cultured neurons, Roy found that populations of known SCb proteins (tagged to photoactivable-GFP) move with properties similar to diffusion, but with a slow bias in the anterograde direction in the axon. The overall motion appears to be microtubule dependent, and to involve intricate particle kinetics together with transient assembly into higher-

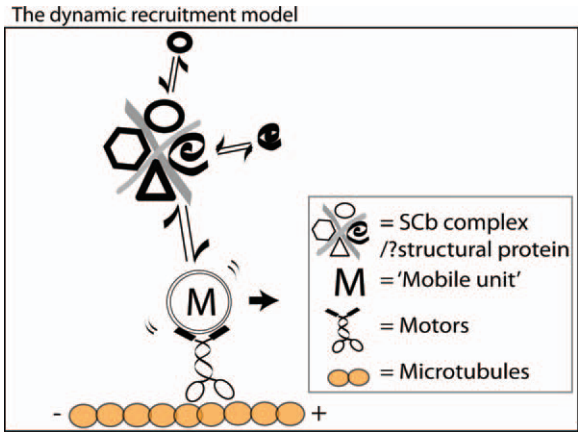

Fig. 5. A hypothetical scenario for SCb transport – the dynamic recruitment model. While proteins with membrane-spanning/anchoring domains are conveyed rapidly as vesicles moving in FAT, proteins lacking such domains (termed cytosolic) are known to move much more slowly, in a group called SCb. The molecular basis for SCb movement is unknown. Based on recent studies, Roy has proposed a model where individual cytosolic molecules assemble into protein complexes that are composed of diverse SCb proteins. Roy's group posited that the SCb molecules associate with structural elements in the axon – perhaps actin, also known to move in SCb – facilitating their collective assembly; and that this structure – along with the assembled cytosolic proteins – then associates with motors. This motor-complex interaction may either be direct; or the complexes may tether to some other mobile organelle within the axon (a hypothetical mobile unit termed M in the figure). In this model, the overall slow movement of the SCb population is a consequence of either the dynamic assembly/disassembly of the cytosolic molecules into complexes; and/or the transient interactions of the complexes with the motor/mobile unit. Roy's group envisions that over time, these transient associations create a slow, biased movement of the entire SCb population. Figure provided by Subhojit Roy.

order structures, reversals and short-range vectorial spurts. Based on these data (together with molecular modeling), Roy proposed that soluble molecules dynamically assemble into supramolecular structures that are transported by motor proteins along microtubules. This proposal is shown schematically in Fig. 5.

To accentuate the fact that the field remains controversial to this day, the session also included a presentation by Jaime Alvarez (Catholic University of Chile). Alvarez asserted that traditional thinking about slow axonal transport may be flawed in the sense that it presumes no local synthesis of proteins in the axon and minimal degradation of proteins. This served as a reminder that neurons are complex cells with multiple intersecting mechanisms that should not be considered in isolation of one another.

Not unexpectedly, the slow axonal transport session included a great deal of robust discussion. For example, Roy was asked why relatively low doses of antimicrotubule drugs suppressed his observed SCb movements, even at concentrations too low to markedly diminish microtubule mass. There was also great interest in how actin is transported, which was not addressed by the studies of any of the speakers. The variety of interesting questions, many of which could not yet be answered with authority, served as an illustration to the students that there is much left to do in even the most fundamental arenas of neuronal cell biology.

Posters by Graduate Students

Posters prepared by graduate students were featured throughout the meeting, with a special session devoted to presentation of the posters. Most of them were related to the topics covered by the oral sessions. Linked to the microtubule session, regulation of cellular properties by cytoskeleton proteins was addressed in the work of Maria Eugenia Chesta (Universidad Nacional de Cordoba, Argentina). She analyzed how post-translational modifications of tubulin were linked to changes in the activity of the Na^+/K^+ ATPase pump. Linked to the neurodegeneration and synaptic sessions, Anthony Ariza (Universidad Cayetano Heredia, Peru) showed how a neuropeptide is differentially distributed in an Alzheimer's-like neurodegenerative disease. Laurie Minamide (Colorado State University) proposed that dimers of amyloid beta peptides, the most synaptotoxic form extracted from human Alzheimer brain, arise through tyrosine oxidation. After oxidation, this human soluble form of Aβ is a potent inducer of cofilin-actin rods. Alzheimer's disease is also characterized by abnormal Cdk5 activity, and building on this, Fredy Castro-Alvarez (Universidad de Antioquia, Colombia) presented evidence that local inhibition of Cdk5 by shRNA-encoded lentivirus attenuated some of the cellular and molecular dysfunctions in a genetic model to study Alzheimer's. Neurodegeneration stimuli found in Parkinson's disease were correlated in a study by Irmgard Paris (Universidad de Chile) with the dysfunction of microtubules and actin microfilaments. Michael Cahill (Northwestern University) showed that the signaling pathway involving Neuregulin 1 and erbB4 induced dendrite growth of interneurons, in a mechanism dependent on the Kalirin protein. This work suggested a correlation between interneurons dendrite development and schizophrenia.

The normal development and function of dendrites were analyzed in several posters. Fernando Bustos (Universidad Andres Bello, Chile) showed how enzymes regulating histone post-translational modification control the expression of scaffold protein at the postsynaptic compartment, thereby regulating dendrite dynamics. Gonzalo Quassollo (Instituto Mercedes y Martin Ferreyra, Argentina) analyzed how signaling proteins linked to actin regulators are at the foundation of the molecular mechanisms controlling dendrite development. Jorge Parodi (Pontificia Universidad Catolica de Chile) presented evidence for a role of Wnt signaling in the control of synaptic activity,

and his work was complemented by that of Macarena Rojas-Abalos (Pontificia Universidad Catolica de Chile) showing the importance of potassium channels in neuronal dendrite changes induced by Wnt signaling.

The poster session also included several presentations related to the establishment and development of neuronal polarity. Work presented by Daniel Borquez (Universidad de Chile) suggested a correlation between controlled protein degradation and the establishment of neuronal polarity. Yasuhiro Funahashi (Nagoya University) discussed how the protein kinase extracellular signal-regulated kinase (ERK), through phosphorylation of a scaffold protein, could potentially modulate the transport of the polarity complex to the nascent axon. A linkage between microtubule and actin microfilaments was suggested in the work of Daniel Henriquez, implicating MAP1B and Tiam1 proteins (Universidad de Chile). Related to MAP1B function, David Villarroel (Universidad de Chile) described how a novel interaction between MAP1B and a p53-modifying enzyme might affect axonal elongation. A novel role for heat shock protein binding partners as neurite outgrowth promoters was presented by Ramiro Quinta (Universidad de Buenos Aires, Argentina).

After neuronal polarization is under way, it is assumed that most of the proteins targeted to the axon are synthesized at the cell body and are then transported by anterograde molecular motors. However, there is also growing evidence that local protein synthesis may occur distally from the cell body, in the axon itself. The work presented by Lucia Canclini (Instituto Clemente Estable, Uruguay) supported this view by showing how transference of mRNA granules from glial cells to neurons could serve as a supply of transcripts to allow local axonal protein synthesis. The conversation between glial cells and neurons may not always be positive for axonal elongation. Secreted proteins from glial cells are potent inducers of neurite collapse. Jose Wojnacki (Instituto Mercedes y Martin Ferreyra, Argentina) showed how the myelin-associated glycoprotein induced changes in the activity of a RhoA-activating factor, leading to neurite collapse. Polarized neurons are able to navigate during nervous system development in response to extracellular cues. Jose San Miguel (University of Minnesota, USA) showed how an actin-nucleating factor is essential to promote growth cone steering of DRG neurons. Lorena Varela-Nallar (Pontificia Universidad Católica de Chile) also analyzed how extracellular cues may modulate neuronal differentiation, using hippocampal neurons as a system model. She described how the Wnt/Frizzled signaling controlled the development of neuronal polarity.

Closing Remarks

Virtually all participants, including the students, attended the business meeting. Discussion was vibrant and enthusiastic. Plans for future meetings began to take root, including both the logistics, as well as ideas to foster even greater interaction among the participants. In particular, there was strong interest in developing ideas for making the meeting even more beneficial to students, for example, through round-table discussions with the established scientists. There was broad agreement that the meeting was highly successful and enjoyable, and should be held regularly in the future. Plans were initiated for the special issue of *Cytoskeleton*, as well as future strategies for funding, and electing new leadership.

And now for the science – we invite you to enjoy the following assortment of peer-reviewed research articles, review articles, and views articles contributed by workshop participants to the "Emerging Concepts in Neuronal Cytoskeleton" special issue, which is being published by *Cytoskeleton* (CSK). Enjoy!

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