became clear that the HII regions targeted in many of the earlier observations were not ideal either for detecting $^3He^+$ or for the modeling required to derive $^3He/H$ (*16*). One of the main difficulties arises from the radiation from bright HII regions, which reflects off the radio telescope superstructure. There are many low-density HII regions (*9*) that are better targets. Reliable abundances determined for a greatly increased sample of HII regions (see the first figure) confirmed that there was no substantial variation with position in our galaxy. The most accurately determined $^3He/H$ abundances were all roughly $(1.9 \pm 0.6) \times 10^{-5}$ (*13*, *17*).

At the same time, improvements in stellar models showed how ^3He production could be almost completely suppressed (*18*, *19*). Rotationally driven mixing could destroy the ^3He produced earlier in the life of a star by mixing ^3He into the deeper, hotter regions of these stars, where it would be fused to ^4He. Mixing would not occur in stars with very low rotation rates, explaining the $^3He/H$ found in some planetary nebulae. Mixing would also produce changes in other abundances, particularly the carbon isotope ratio, $^{12}C/^{13}C$. This ratio can be measured in stars, enabling statistics to be collected on what fraction of stars do not mix and thus enrich the interstellar medium in ^3He. Taking these factors into account in models for the chemical evolution of the Milky Way yielded abundances that began to resemble observations (*20*).

^3He is now well enough understood to be used as a cosmological probe (*17*). D, ^3He, ^4He, and the fluctuations in the cosmic microwave background all suggest that the amount of ordinary baryonic matter (made of protons, neutrons, and electrons) in the universe is only about 4% the amount that would be required to reverse the expansion of the universe.

Out with the old. The National Radio Astronomy Observatory 140 Foot Radio Telescope during its last scientific observing run, July 1999. The Robert C. Byrd Green Bank Telescope is seen during construction in the background.

We are only beginning to understand the evolution of ^3He within the Milky Way. In the next few years, observations of ^3He should be greatly facilitated by the Robert C. Byrd Radio Telescope now undergoing commissioning in Green Bank, WV (see the second figure). Its design should eliminate the superstructure reflections that limited earlier observations. The currently observed sample of planetary nebulae is highly biased. Increasing the sample size with the new telescope should allow the consequences of this bias to be evaluated. The HII region survey can be extended to a larger fraction of our galaxy than for any other isotope. ^3He could well become the most widely used constraint to models of the chemical evolution of our galaxy.

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PERSPECTIVES: BIOMEDICINE

A Cargo Receptor Mystery *APP*arently Solved?

Sangram S. Sisodia

Alzheimer's disease (AD), the most common neurodegenerative disease of old age, is characterized by deposits of small amyloid β (Aβ) peptides in the brain. These Aβ peptides are produced by proteolysis of a type I integral membrane protein, the amyloid precursor protein (APP), by the enzymes BACE1 and γ-secretase. BACE1, which generates the amino terminus of Aβ, is a membrane-tethered aspartyl protease (*1*). The identity of γ-secretase, which is responsible for cleaving the transmembrane region of APP and liberating Aβ peptides, is controversial (*2*). However, the presenilins (PSs), a highly conserved family of serpentine membrane proteins, are essential for facilitating this reaction.

Little is known about the subcellular distribution of BACE1 and PS, and it is here that the recent *Nature* report by Goldstein and colleagues offers provocative insights (*3*). These investigators show that BACE1 and PS are found in membrane vesicles transported along the axons of peripheral and central mouse neurons, and that this transport requires APP. These cargo-laden membrane vesicles are bound to a motor protein complex, kinesin-1, which is composed of two components (see the figure). The first is a kinesin heavy chain (KIF5B) containing ATP- and microtubule-binding motifs that is essential for vesicle transport. The second component, called kinesin light chain (KLC), associates with KIF5B and tethers membrane vesicles containing a subset of proteins that are transported along the axon from the neuronal cell body to nerve terminals (*4*) (see the figure). The transported proteins (cargo) include the neurotrophin receptor TrkA, the synaptic vesicle–associated phosphoprotein synapsin 1, and the growth-associated protein GAP-43, which regulates cytoskeletal dynamics in neuronal growth cones. Most surprising is the finding by Goldstein's lab that Aβ peptides can be generated within axons, and within isolated membrane vesicles from sciatic nerves. The authors offer the tantalizing proposal that in disease set-

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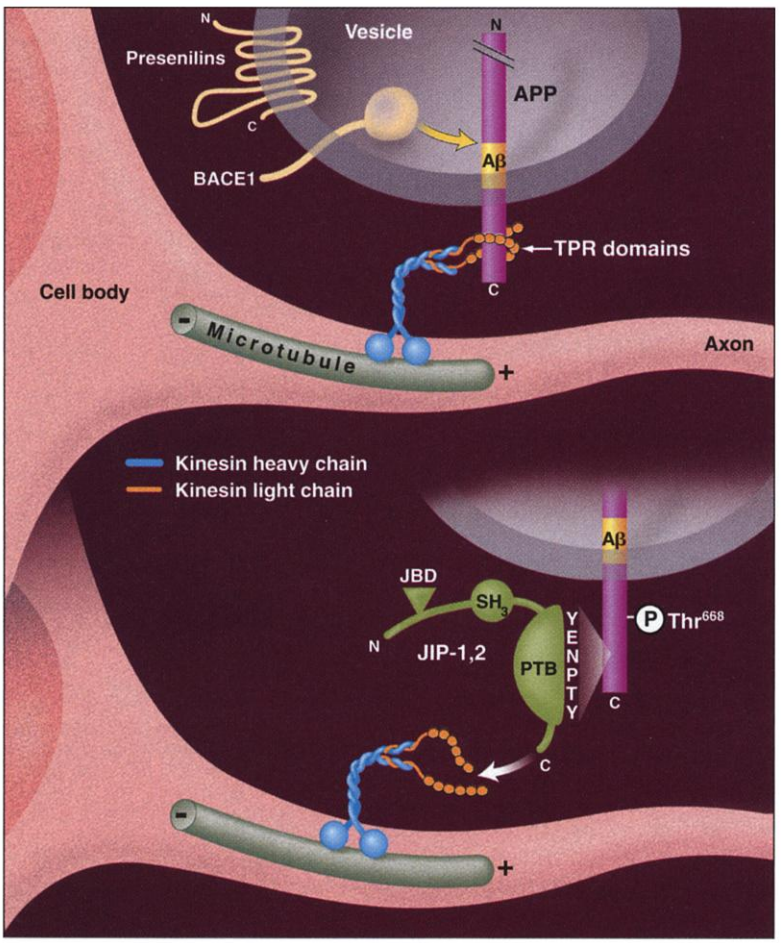
tings, Aβ deposition in axonal membrane vesicles could effectively deprive neuronal cell bodies of growth and survival factors, ultimately leading to neurodegeneration. Their evidence, taken together with earlier work (*5*) showing that APP in the membrane vesicle is a receptor for kinesin-1, has fueled debate in the AD community and among the motor protein cognoscenti alike.

A central tenet of the Goldstein work is that APP is required for kinesin 1–dependent transport of the enzymatic machinery responsible for Aβ production. It is not surprising that BACE1, normally resident in the trans-Golgi network, could be transported along axons. In contrast, it is a mystery how PS could be redistributed from membrane vesicles in neuronal cell bodies and dendrites of embryonic neurons (*6–8*) to axonal membrane vesicles in the adult. Not in question, however, is the fact that APP is transported along axons to nerve terminals (*9–11*), and that transport of APP depends on kinesin-1 (*12*, *13*).

Unfortunately, and despite evidence supporting a multiplicity of roles for APP—including neurite outgrowth, neuroprotection, and activation of MAP kinases and K^+ channels (*14*)—it is not clear what APP does in neurons. APP is a member of a larger family of proteins that includes amyloid precursor-like proteins 1 and 2 (APLP1 and APLP2), which lack the Aβ domain. Mice engineered to be deficient in either *APP*, *APLP1*, or *APLP2* are viable with very subtle deficits. In contrast, mice lacking two of the three proteins (*APP* and *APLP2*, or *APLP1* and *APLP2*) die shortly after birth but without any obvious structural abnormalities in the brain (*15*, *16*). In view of the rather modest effects of APP and APLP loss on neurons, it is surprising that Goldstein and co-workers (*5*) found that APP is a receptor for kinesin-1. These authors document that an APP fusion protein binds to the tetratricopeptide repeat modules of kinesin light chains (KLC-1 and KLC-2) with high affinity (~15 to 20 nM), and furthermore, that there is a reduction in the amount of APP and GAP-43 transported along peripheral sciatic nerves in mice lacking KLC-1. Complementary studies revealed that deletion of the *APP-like* gene (*Appl*) in the fly (*17*), or overexpression of human APP constructs that contained APP or APLP2 cytoplasmic domains, caused aberrant accumulation of transported membrane vesicles within axons. This phenotype has also been observed in flies that lack kinesin or dynein, a microtubule-activated ATPase that transports membrane vesicles from neuronal dendrites back to the cell body (*17*, *18*).

Collectively, these findings would imply that APP is the receptor for kinesin-1. On the other hand, the observation that transport of BACE1, PS, KIF5B, and KLC still occurs, albeit at lower levels, in APP-deficient mice, suggests that APP is unlikely to be the only cargo receptor for kinesin-1. It is possible that axonally transported APLP1 and APLP2 (*3*) might compensate for APP. Also, a family of proteins—JNK-interacting protein 1 (JIP-1), JIP-2, and JIP-3—bind to the tetratricopeptide domains of KLC and serve as scaffolds for JNK and associated MAP kinases, as well as for signaling receptors including the reelin receptor and ApoER2 (*19*). JIP-3 is a homolog of Sunday Driver (SYD) in the fly (*20*) and UNC-16 in the worm (*21*). Reducing SYD or UNC-16 expression results in accumulation and redistribution of synaptic vesicles in neuronal axons.

Despite the high-affinity binding of APP and KLC in vitro, there is no evidence as yet that APP and KLC directly associate with each other in vivo. Enter JIP-1, which harbors a domain that binds to phosphorylated tyrosine residues (*22*, *23*). In neurons, a specific threonine residue (threonine 668, or Thr^{668}) in the APP cytoplasmic domain is phosphorylated by cyclin-dependent kinase 5 (Cdk5) (*24*). Phosphorylated APP is selectively localized within neuronal growth cones and neurites (*25*). Muresan *et al.* (*26*) recently reported that a mutant version of APP lacking Thr^{668} failed to be transported along the neurites of cultured neurons, and that APP phosphorylated in vitro at Thr^{668} by Cdk5 preferentially interacts with KLC in these neurons. This study suggests that Cdk5-mediated phosphorylation of APP's Thr^{668} might promote the association of APP with KLC (see the figure). Given that APP can associate with JIP-1, it is possible that phosphorylation of APP at Thr^{668} could enhance its affinity for JIP-1, and result in the subsequent recruitment of the APP–JIP1 complex to kinesin-1 (see the figure). Intriguingly, phosphorylated APP carboxyl-terminal fragments also accumulate at nerve terminals in the brain (*11*). Thus, it remains



The association of APP with kinesin-1. Membrane vesicles containing protein cargo are transported along axons from the neuronal cell body to nerve terminals. This process is dependent on kinesin-1, which consists of two kinesin heavy chains (KHCs) (blue) and two kinesin light chains (KLCs) (orange). The KLCs are bound to axonal microtubules via the microtubule- and ATP-binding domains of KHC. There are two possible ways in which membrane vesicles containing cargo can associate with kinesin-1. In the first, APP binds directly to the tetratricopeptide (TPR) domains of KLCs, thus linking membrane vesicles to microtubules. In the second, APP or APP carboxyl-terminal fragments could bind to the phosphotyrosine-binding domains of the JIP scaffolding protein family. The JIPs in turn could then bind to the TPR domains of KLC via their carboxyl-terminal regions. In addition, phosphorylation of Thr^{668} in the APP cytoplasmic domain might serve to modulate the interaction of APP and APP carboxyl-terminal fragments with the KLCs or JIPs.

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possible that, like APP, these fragments are generated by BACE1 in the trans-Golgi network and might also serve to tether membrane vesicles to KLCs, a model that can now be tested by examining whether expression of APP carboxyl-terminal fragments in the neurons of APP-deficient mice can rescue axonal transport abnormalities in these animals.

Goldstein and colleagues observed that Aβ can be generated in membrane vesicles transported along the axons of peripheral nerves. This finding needs to be confirmed in brain neurons (*11*). In any event, these authors speculate that impaired APP transport leads to enhanced axonal generation and deposition of Aβ, resulting in disruption of neurotrophic signaling and neurodegeneration. Although attractive, there is limited evidence in humans, or in mouse models, to support this notion. Aβ deposits are rarely seen in the deep white matter of human brains, and dystrophic or swollen axons are present only in the vicinity of amyloid deposits. Furthermore, the accumulation of membrane vesicles within axons is not apparent in either young or aged transgenic mice that have 8- to 10-fold higher levels of human APP containing mutations that cause familial AD. A closer examination of axonal transport and axonal pathology in these animals is warranted.

Finally, the notion that APP is a kinesin-1 receptor overlooks the possibility that APP has a job at the synapse, and that γ-secretase–generated APP carboxyl-terminal fragments have transcriptional activity (*27*). A model that accommodates these alternative activities posits a dual role for APP: as a kinesin-1 receptor that facilitates the delivery of specific cargo proteins to specialized presynaptic sites, and as a receptor/ligand at nerve terminals. In this model, fusion of the transported vesicles with the presynaptic plasma membrane "exposes" the membrane-bound APP to specific ligands, resulting in activation of intracellular signaling events or the release of carboxyl terminal–truncated APP and Aβ into the synaptic cleft.

Stay tuned for more findings from this exciting foray into the biology of APP and APLPs in axonal transport and synaptic activity. These efforts will provide new insights into the molecular apparatus that regulates kinesin-1 selection of particular cargo vesicles and axonal trafficking. Such research will open the door to understanding how disruptions of these pathways might affect the initiation or progression of age-associated neurodegenerative diseases, such as AD.

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PERSPECTIVES: TRANSCRIPTION

Oxygen Sensing Gets a Second Wind

Richard K. Bruick and Steven L. McKnight

Mammalian cells are able to sense prolonged decreases in oxygen concentration (hypoxia) through a conserved hypoxic response pathway. This pathway facilitates adaptation to hypoxia-induced physiological stress by regulating changes in gene expression, and is also critical for the execution of many physiological events, including formation of blood vessels during embryogenesis, and pathophysiological processes such as tumorigenesis. A family of hypoxia-inducible transcription factors (HIFs) lies at the heart of this adaptive pathway. HIF proteins are activated by a decrease in the concentration of molecular oxygen (O_2), which results in the induced expression of downstream target genes that mediate adaptation and survival of cells and the whole organism.

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Until recently, the means by which cells sense alterations in oxygen tension and subsequently induce changes in HIF activity remained obscure. The first inkling of an oxygen sensing pathway in higher organisms came last year with the discovery of a family of oxygen-dependent enzymes responsible for the modulation of HIF stability (*1*–*4*). A report by Lando *et al.* (*5*) on page 858 of this issue now identifies a second, oxygen-dependent, posttranslational modification of HIF that regulates the ability of HIF to recruit stimulatory transcriptional cofactors to its target genes.

The HIF transcription factors are composed of two subunits: the hypoxia-regulated α subunit, HIF-1α (or its paralogs HIF-2α and HIF-3α), and the oxygen-insensitive HIF-1β subunit (also known as the arylhydrocarbon receptor nuclear translocator, or ARNT). Under normal oxygen conditions (normoxia), the HIF-1α subunit, although expressed, is rapidly degraded such that almost no HIF protein accumulates (see the figure). Under hypoxic conditions, degradation of the α subunit is blocked, allowing HIF-1α to accumulate within the nucleus where, upon binding to HIF-1β, it recognizes HIF-responsive elements (HREs) within the promoters of hypoxia-responsive target genes. Degradation of HIF-1α under normoxic conditions is triggered by posttranslational hydroxylation of conserved proline residues within a polypeptide segment known as the oxygen-dependent degradation domain (ODD). The hydroxylated proline residues in this sequence are recognized by the product of the von Hippel–Lindau tumor suppressor gene (pVHL), a component of a ubiquitin ligase complex that tags the α subunit for degradation by the proteasome (see the figure) (*6*, *7*). This critical regulatory event is carried out by a family of iron (II)–dependent prolyl hydroxylase enzymes (*3*, *4*) that use O_2 as a substrate to catalyze hydroxylation of the target proline residues. Because O_2 appears to be rate limiting for prolyl hydroxylase activity (*3*), these enzymes may represent bona fide oxygen sensors that provide a direct link between O_2 concentration and components of the hypoxic response pathway.

Modulation of protein stability is just one means by which HIF activity is induced by hypoxia. In addition to the ODD domain, the α subunits of all three HIF isoforms contain two transactivation domains responsible for recruiting transcriptional coactivators essential for gene expression. One of the HIF transactivation domains overlaps the ODD, and regulation of its activity is likely to be a by-product of protein