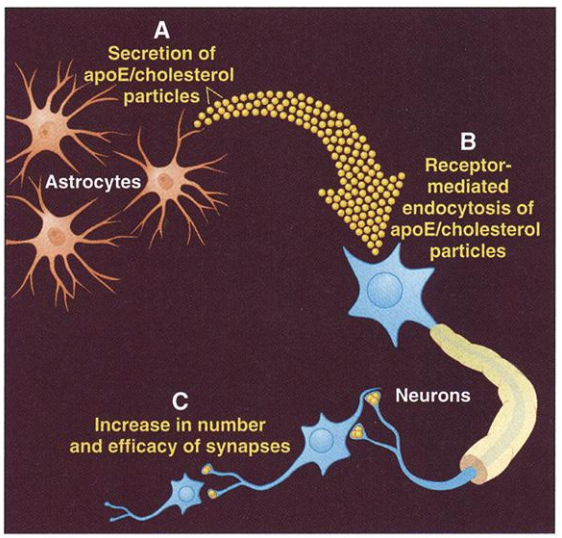
PERSPECTIVES: NEUROBIOLOGY

Cholesterol—Making or Breaking the Synapse

Ben A. Barres and Stephen J. Smith

The smooth operation of the nervous system depends on rapid communication between nerve cells at meeting areas called synapses. Although synapses were first identified 100 years ago, their formation, a process called synaptogenesis, has remained something of a mystery. For example, it is still not clear how many synapses a neuron can make with other nerve cells. Is the number of synapses rigidly preprogrammed or is it plastic, governed by interactions with neighboring cells? Thanks to a simple model system in which a defined type of neuron from the central nervous system is purified and cultured, two intriguing but unanticipated conclusions about synaptogenesis have been reached. The first is that neurons by themselves form few synapses unless they have help from other nerve cells called glial cells (*1*–*3*). The second, reported by Mauch *et al.* (*4*) on page 1354 of this issue, is that the synapse-promoting signal released by glial cells is cholesterol.

The story begins 4 years ago when Pfrieger and Barres (*1*) asked whether purified retinal ganglion cells would form functional synapses in culture. They obtained these neurons from postnatal rat retinas at a purity of greater than 99.5% with an antibody-selection process called immunopanning (*5*). They kept their purified retinal ganglion cell cultures alive in a simple serum-free culture medium that prevented most of the cells from undergoing apoptosis despite the absence of a subpopulation of glial cells called astrocytes that secrete factors promoting neuronal survival (*6*). To their surprise, the retinal ganglion cells exhibited little synaptic activity unless they were cocultured with astrocytes, in which case spontaneous synaptic activity increased 70-fold. To further investigate exactly how these glial cells could so powerfully control synaptic activity in these cultures, the researchers next performed electrophysiology, imaging, immunostaining, and electron microscopy (*2*, *3*). They found that astrocytes increased the total number of synapses on each neuron by sevenfold. Moreover, astrocytes were required to maintain these synapses, because most synapses formed in the presence of astrocytes were quickly lost when these cells were removed (*2*, *7*). These studies suggested that glial cells, long thought to be passive bystanders in the formation and operation of our neural circuitry, might actively participate in the making and breaking of synapses.



Cholesterol—making a nervous debut. Neurons in culture form few synapses unless glial cells called astrocytes are present. **(A)** Astrocytes increase synapse number by secreting cholesterol bound to large lipoprotein particles containing apolipoprotein E (apoE). **(B)** These particles are internalized by neurons, leading to increased cholesterol within neuronal membranes. It is possible that apoE also activates yet to-be-identified signaling pathways within the neurons. **(C)** These changes stimulate an increase in the number and efficacy of synapses.

These findings also raised an important question: How do glial cells control the number of synapses? Early work had shown that the astrocyte-derived signal was released into the medium as a soluble protein (*1*). To identify the signal, Mauch and colleagues fractionated astrocyte-conditioned medium and found an activity with a large molecular weight (150 to 650 kilodaltons) that could bind to heparin (*4*). They further showed that astrocytes reliably induced the appearance of a protein in neurons, which they identified, by microsequencing and mass spectrometry, as apolipoprotein E (apoE). ApoE is a heparin-binding constituent of the many large lipoprotein particles that transport lipids between cells and around the body. In the brain, apoE is primarily made by astrocytes, and apoE receptors are abundantly expressed by neurons. This suggested to Mauch and coworkers that neurons might take up and accumulate apoE secreted by astrocytes and, thus, that apoE might be the glial-derived synapse-promoting signal they were looking for. Recombinant apoE, however, did not induce the purified neurons to form synapses. Because apoE-containing lipoproteins are cholesterol carriers, the investigators next examined the effects of adding cholesterol to the culture medium. Amazingly, cholesterol by itself induced the neurons to form almost as many synapses as did medium enriched for factors secreted by astrocytes. Moreover, removing cholesterol from this medium or inhibiting cholesterol synthesis abrogated the ability of astrocytes to promote synapse formation. Although the authors did not test whether depletion of apoE from the medium would have a similar effect, they found that a general antagonist of lipoprotein receptors significantly decreased the ability of astrocytes to trigger synapse formation. The simplest explanation for these findings is that cholesterol bound to apoE-containing lipoprotein particles is released by astrocytes and taken up by neurons, where it then promotes an increase in synapse number. Consistent with this possibility, neurons cultured in the presence of astrocytes contain twice as much cholesterol within their membranes as neurons that are cultured alone.

The new findings raise many important questions. First, is apoE itself the astrocyte-derived protein responsible for increasing synapse number? It remains possible that another apolipoprotein or other yet to-be-identified glial proteins are involved. Second, which of the many low-density lipoprotein (LDL)–related receptor family members that bind to apoE mediate this effect on synapse formation? Although some of these receptors simply aid in the taking up of lipoproteins, others have been implicated in signal transduction (*8*). This raises the interesting issue of how exactly cholesterol increases synapse formation. One possibility is that cholesterol is needed to activate a signaling pathway that triggers synaptogenesis—either an apoE receptor pathway or another signaling pathway such as the sonic hedgehog, Wnt, or reelin cascades (*8*, *9*). Alternatively, a sufficient amount of choles-

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terol itself might be needed to support the structural demands of synaptogenesis. For example, cholesterol binds to several synaptic proteins, and is necessary for the formation of synaptic vesicles and for the clustering of certain postsynaptic receptors (*10*–*12*).

But perhaps the most important question is whether the glial delivery of cholesterol to neurons within the brain is the limiting factor regulating synapse formation. Cholesterol within the brain is derived almost entirely through in situ synthesis by brain cells (*13*). The appearance of most synapses in the developing brain is temporally and spatially coincident with the development of astrocytes, suggesting that synapse formation may depend on astrocyte-derived cholesterol (*2*). It is possible that once astrocytes begin to develop, neurons always have plenty of cholesterol available to them in vivo. On the other hand, glia have recently been found to control synapse number within the developing cerebellum of transgenic mice whose glia express mutant glutamate receptors (*14*). These findings are consistent with the possibility that astrocytes, by providing a limiting cholesterol supply to neurons, control synapse formation in vivo.

Could the cholesterol supply also regulate synaptic plasticity in the adult brain? Although astrocytes are needed to maintain synapses formed in culture, it is not yet clear whether cholesterol is similarly required. The LDL receptor–related protein (LRP), however, has been directly implicated in synaptic plasticity in hippocampal slices (*15*). Even more intriguing, apoE has long been suspected to be involved in neurodegenerative loss of synaptic plasticity in Alzheimer's disease (*16*). The apoE4 isoform is associated with an increased risk of late-onset Alzheimer's disease and is less able to promote neurite outgrowth than other apoE isoforms (*17*). Will apoE4 also differ in its ability to promote synapse formation? Fortunately, identification of cholesterol as the glial-derived synapse-promoting signal should make it possible to investigate the involvement of cholesterol and glia in synaptic development and plasticity in vivo.

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PERSPECTIVES: GEOLOGY

Deep Below North America

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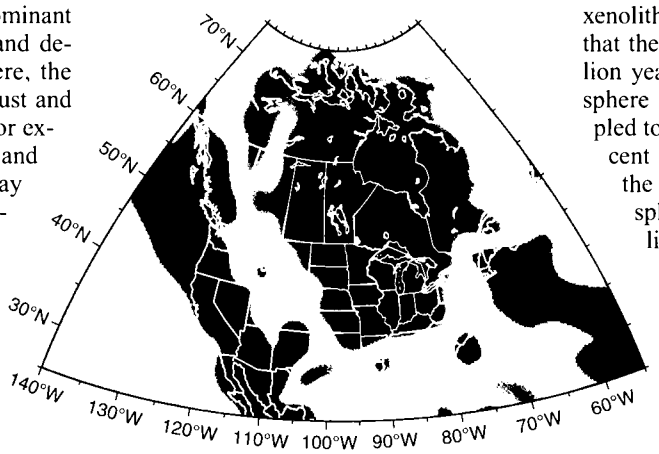
Today, plate tectonics is the dominant process behind the creation and destruction of Earth's lithosphere, the stiff outer layer that includes the crust and the uppermost part of the mantle. For example, as the South American Plate and the African Plate are moving away from one another, lithosphere is created in the central South Atlantic and begins to be destroyed in the subduction zone beneath the Andes. Was plate tectonics also a dominant process in Earth's early history? To answer this question, we must study the oldest parts of today's lithosphere.

The oldest rocks on Earth are found in the Slave Province, a geological region in the Northwest Territories, Canada. These rocks formed 4030 million years ago (*1*, *2*)—only about 500 million years after the formation of the solar system and 3900 million years before Gondwanaland broke up into Africa and South America. Practically all rocks and geological structures of the Slave Province are older than 2500 million years, and the area has been tectonically undisturbed ever since (*3*).



Geophysical map of North America. The blue and red shades are a proxy for the stiffness of mantle rocks at a depth of 140 km; blue represents the stiffest material. This map was produced as part of an ongoing collaboration (*5*, *6*) and is based on the analysis of over 1200 seismograms from different seismographic networks in North America. The large blue region roughly represents the part of North America that is older than 1000 million years and is called Laurentia.

Geophysical observations indicate that North American Provinces that are older than 1000 million years, including the Slave Province, generally have a cold, stiff lithosphere (*4*) that is 250 km thick on average (*5*, *6*). Some rocks, called mantle xenoliths, have been brought to the surface from deep within this thick lithosphere by rapidly ascending gas-rich magmas. In the Slave Province, these magmas also carried diamonds to the surface, suggesting that the deep lithosphere could be as old as the crust. Indeed, recent analyses of mantle xenoliths from the Slave Province indicate that the deep lithosphere is over 2500 million years old (*7*, *8*). This old, deep lithosphere has thus remained physically coupled to the overlying crust during more recent plate tectonic processes, leading to the widespread use of the term tectosphere (*4*) for such thick, continental lithosphere.

The tectosphere is characterized by relatively low temperatures because it has not participated in mantle convection for hundreds to thousands of millions of years. Furthermore, it is relatively depleted in iron (*4*), preventing it from sinking into the deeper mantle. The low temperature causes the tectosphere to be stiffer than the average upper mantle, resulting in faster propagation of seismic waves through the tectosphere. Seismic waves that traversed the continent can be combined into a three-dimensional map of seismic wave speeds, which may be considered to first order as a proxy for stiffness—and temperature (*9*)—of the North American upper mantle (see the figure).

The large blue area in the figure is roughly coincident with Laurentia (*3*, *10*), the predecessor of the present North American continent. Laurentia consists of a large

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