QNAS

QnAs with Ben Barres

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The density of cells in the human brain defies imagination. Among the cellular throng, neurons garner much of the credit for brain function. Viewed under the microscope, however, slices of brain tissue are crisscrossed by swarms of cells of varying shapes. Collectively called glia, these cells enrobe neurons, embrace blood vessels, envelop synapses, and alter the chemical milieu in which the brain is awash. As collaborators in brain function, glial cells were once considered a mildly fascinating sideshow but have recently benefited from a revival of intense scientific interest. Ben Barres, a neuroscientist at Stanford University and the first transgendered researcher to be elected to the National Academy of Sciences, has devoted a distinguished career to solving what he eloquently puts as "the mystery and magic of glia." Over the years, Barres' pursuit has helped redefine the scientific understanding of his favored cell type: Far from being a janitorial workforce, glia, it turns out, control how synapses form, function, and fade away, suggesting that these resourceful cells might play a prominent role in activities commonly ascribed to neurons. The therapeutic implications of these findings are not lost on Barres, who explores the potential of glia as drug targets in brain diseases. Barres recently spoke to PNAS about his interest in glia.

PNAS: Over two decades, you have helped revise the false notion of glia as the nervous system's lowly foot soldiers, whose *raison d'être* is to serve and support neurons. When did you become interested in glia?

Barres: I became interested in glia during my neurology training. As residents, we would look at diseased brain slices from patients under the microscope. I was hooked by the dramatic morphological changes happening in glial cells in injured or diseased brain tissues. Glia, astrocytes in particular, send out processes that make contact with major blood vessels, but most astrocytes are clustered around synapses. Back then, it was unclear what astrocytes did in the brain, and the prevailing view was that they were passive support cells for neurons. We purified neurons and glia and studied their interactions in culture dishes, following the tradition of immunologists, who had previously isolated different types of immune cells to study their functions and interactions. We found that astrocytes are not so passive after all: In culture, they are polarized, electrically excitable, and make axons and dendrites, just like neurons do. But they cannot form synapses on their own. Importantly, our ability to grow neurons and astrocytes separately allowed us to make the surprising discovery that astrocytes secrete signals that are crucial for synapse formation, maintenance, and function.

PNAS: More recently, you expanded the panoply of functions for astrocytes with another finding: Astrocytes participate in synapse elimination. Can you describe the significance of this role?

Barres: During development, the brain overproduces synapses, and the excess synapses are pruned during a window of development. Previously, we had shown that complement proteins [innate immune system proteins involved in clearing pathogens and cellular debris] are associated with synapses throughout the central nervous system during the synapse-pruning window. That finding suggested that complement might play a role in synapse elimination, analogous to its immunological role in eliminating pathogens. Synapses coated with complement proteins are recognized by microglia, which then clear the synapses. After brain development is completed, the complement cascade is turned off, presumably because the pruning is finished and circuits are stabilized. (As an aside, the complement cascade turns back on in neurodegenerative diseases like Alzheimer's. So the untimely reactivation of synaptic pruning in the adult brain might contribute to such diseases.) We used the mouse retinogeniculate circuit, which is an elegant experimental system to track synapses. In this circuit, we showed that astrocytes also actively engulf and eliminate synapses in a manner dependent on neuronal activity but independent of the complement proteins. We identified two other phagocytic pathways that the astrocytes use to prune synapses.

PNAS: So you are suggesting that neuronal activity-dependent changes that occur during learning and memory might in fact be tied to astrocytes.

Ben Barres.

Barres: Yes, we strongly suspect so. We are now exploring whether the critical window of developmental plasticity, when the brain undergoes early synaptic pruning, might be under the control of an "astrocyte clock." So it is possible—and this is our novel and intriguing hypothesis—that neuronal activity-dependent synapse pruning is in fact tied to the activation of astrocytes and their ability to gobble synapses. After all, astrocytes can detect many kinds of neuronal signals, including neurotransmitters.

PNAS: Have your findings on the functions of glia led to the discovery of molecular drug targets?

Barres: We have shown that astrocytes secrete a signal called thrombospondin, which is necessary for synapse formation, and that astrocytes also have a receptor for thrombospondin. The pharmaceutical industry has been largely focused on neurons, but our finding suggests that glia might be important drug targets. The thrombospondin receptor

This is a QnAs with a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 10093.

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on neurons is also the receptor for the blockbuster drug neurontin or gabapentin, used to treat pain and epilepsy. By showing that the receptor is necessary for thrombospondin to induce excitatory synapse formation, including synapses in pain circuits, and that neurontin antagonizes this interaction, we have uncovered a potential mechanism of action for the drug. In addition, our findings on synapse pruning demonstrate that the classical complement cascade is an important target for neurodegenerative diseases, such as Alzheimer's disease.

PNAS: In your Inaugural Article (1), you explore the phenomenon of axon degeneration, a common feature of many neurodegenerative diseases and injuries, focusing on the role of a protein that might hold a key to treating such diseases. Can you describe the protein?

Barres: There is a lot of interest in finding drugs to slow or halt axon loss in the central nervous system. Several years ago, it was shown that in mutant mice that produced a chimeric protein called WldS, or Wallerian degeneration slow, which is formed by fusing parts of two different proteins, the axons were protected from degeneration after injury. This protection seems to be conserved in several species; WldS promptly became a potential drug target, but its mechanism was unclear. So we tried to find out whether the protein turns on protective genes in the nucleus of the neuron or acts directly in the axon to protect it from degeneration. To answer that question, my student Jack Wang used a technique developed here at Stanford to control the stability and function of proteins in cells. He found that the activity of WldS is required not in the nucleus of the neurons but in the axons. Even after the axons are severed, if WldS is stabilized in the axons, there is a window of four to five hours after injury when axonal degeneration can be blocked.

PNAS: What do these findings mean for the treatment of nerve damage?

Barres: The implications of these findings are that if you administered a drug that mimics Wlds during this therapeutic window after injury, you might be able to save axons from degeneration. The metabolite NAD is precisely such a drug candidate, and further studies will reveal how it might be used to prevent axon degeneration.

PNAS: Your scientific work adds to the revisionist literature that has helped change researchers' regard for a long-neglected cell type. You have also been a voluble spokesperson for other underrepresented entities in science: women, for example. Would it be overreaching to infer a theme?

Barres: Science proceeds at its best when it includes diverse studies performed by diverse scientists.

1 Wang JT, Medress ZA, Vargas ME, Barres BA (2015) Local axonal protection by Wlds as revealed by conditional regulation of protein stability. *Proc Natl Acad Sci USA* 112:10093–10100.