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Glial Control of Synaptogenesis

Though all communication between neurons occurs through synapses, we know surprisingly little about the mechanisms inducing their formation. In this issue of *Cell*, Barres and colleagues (Christopherson et al., 2005) demonstrate that glial-derived thrombospondins and additional soluble glial-secreted factors regulate synapse assembly and functional maturation.

Making functional synapses is generally thought of as a job for neurons. But could non-neuronal factors promote synaptogenesis? The majority of mammalian synapse formation occurs during early postnatal development, and far fewer synapses are formed in the adult nervous system. Immediately prior to this postnatal wave of CNS synapse construction, huge numbers of astrocytes are generated in the developing brain. This enigmatic cell type is the most abundant in our brain, but for decades astrocytes have been relegated to the role of simple support cells, maintainers of the delicate environment that neurons need to tend to the important business of constructing and running the CNS. A study in this issue of *Cell* by Barres and colleagues (Christopherson et al., 2005) suggests that we should reconsider any views we have of astrocytes as passive spectators in synapse formation. They identify two soluble factors secreted by astrocytes that regulate synaptogenesis. The first is thrombospondin which promotes the formation of morphologically normal but functionally silent synapses; the second (yet to be identified) converts these silent structures to functionally mature synapses.

When purified mammalian retinal ganglion cells (RGCs) are cultured for several days below (but not in contact with) a feeding layer of astrocytes they form 7-fold more functional synapses than RGCs cultured alone (Nagler et al., 2001; Pfrieger and Barres, 1997; Ullian et al., 2001). Thus soluble glial-derived factors can promote the formation of functional synapses. What are these factors? To begin the hunt Barres and colleagues compared the effects of astrocyte feeding layers to astrocyte-conditioned medium (ACM) on synapse formation in RGC cultures. ACM was found to induce morphologically normal synapses at levels similar to astrocyte feeding layers, and they used this ACM-induced increase in RGC synapse number as an assay to track down the synaptogenic molecule present in fractionated ACM. The ACM synaptogenic activity co-purified with fractions >300 kDa and bound heparin. This led the authors to focus on thrombospondins (TSPs), which are normally expressed in glia and present in ACM. TSPs are also oligomeric extracellular matrix proteins (with the complexes exceeding 300 kDa) that bind heparin. Strikingly, they found that purified human TSP1 increased synapse formation to a similar degree as ACM. These TSP1-induced synapses are ultrastructurally normal pre- and postsynaptically when compared to synapses in RGC cultures grown with an astrocyte feeding layer and contain all assayed pre- and postsynaptic structural proteins. TSP1 treatment does not affect total levels of synaptic proteins, indicating that TSP1 is affecting the localization of synaptic proteins to new synapses rather than inducing their expression. TSP2, a closely related TSP family member, was also found to induce synapse formation at levels similar to TSP1, and immunodepletion of TSP2 from ACM reduced the number of induced synapses to control levels.

Are TSPs essential synaptogenic proteins in vivo? TSP is expressed in the brain during postnatal stages when the majority of CNS synapses are forming, and it colocalizes with synaptic markers in multiple brain regions. In addition, TSPs are significantly downregulated in the adult when synaptogenesis is dramatically decreased. Moreover, TSP1 and TSP2 function are essential for promoting synaptogenesis: TSP1/TSP2 double mutant mice exhibit a dramatic reduction in the number of synapses formed during postnatal stages. Thus TSPs are the key synaptogenic signal in ACM, high-level TSP expression coincides with high-level synaptogenesis in vivo, and loss of TSP function significantly decreases synapse formation. These are the first data supporting an in vivo role for glial-derived soluble factors in promoting synaptogenesis. Additionally, these results suggest the exciting possibility that TSP expression may define a window during postnatal developmental when high levels of synaptogenesis can occur.

How do TSPs promote synapse formation? TSPs could act as permissive or instructive cues. The former may be the more likely possibility because of their diffuse distribution within the developing brain. But if TSPs are permissive and required for synapse maintenance in culture, why are they absent from the adult CNS where synapses are stably maintained? Are additional TSP family members acting as stabilizing signals? Identifying the neuronal TSP receptor may help clarify these issues, and there are many candidates as TSPs are known to bind a dizzying array of extracellular matrix molecules and transmembrane receptors (Adams, 2001). Equally important will be exploring potential roles for TSPs in CNS disease and trauma. For example, do reactive glia generated after CNS injury

express TSPs which promote synaptogenesis at the expense of axon growth?

The identification of TSPs as glial-secreted factors that promote synaptogenesis is itself very exciting, but this work also addresses additional mechanisms by which glia can modulate synapse function. Interestingly, while the TSP- and ACM-induced synapses are morphologically normal and presynaptically active, they are postsynaptically silent, lacking their normal AMPA receptor-mediated response. In contrast, synapses induced by astrocyte feeding layers were found to be active both pre- and postsynaptically, having normal AMPA receptor-mediated responses. These data lead to a model whereby glia first secrete TSPs to drive the synapse assembly and subsequently secrete an unidentified second signal that converts these silent synapses into functional units. This silent to active synapse conversion through activation of AMPA receptor-mediated responses is highly reminiscent of the AMPA receptor-dependent enhancement of synaptic strength observed following the induction of long-term potentiation (Isaac et al., 1995; Liao et al., 1995). Therefore the ever-increasing list of essential glial CNS functions may soon extend to modulation of synaptic plasticity. A tantalizing result supports this idea: the capacity for ocular dominance plasticity can be restored in mammalian primary visual cortex by simply injecting immature astrocytes (Muller and Best, 1989). In addition, exciting new work comparing gene expression patterns in brains of humans and other nonhuman primates reveals that one of the major differences is a dramatic increase in TSP expression levels (Preuss et al., 2004). Does this mean we have an inherently greater capacity to form synapses? Can this difference help begin to explain our superior cognitive function?

This work adds tremendously to our understanding of how glia can control synapse formation and maturation. It is now clear that all major aspects of synapse biology—assembly, functional maturation, and efficacy of firing—can be directly regulated by factors secreted by glia. This should force us to rethink the importance of these "support cells" in CNS development and function.

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