association of GATOR1 with RagA (Jin et al., 2015), what mechanism provides the brake to restrict GATOR1 activity at early time points of amino acid stimulation, and how is this brake released at later time points to attenuate mTORC1 signaling? As GATOR2 inhibits GATOR1 (Bar-Peled et al., 2013), perhaps GATOR2 represents the brake? It is important to note that a curious discrepancy exists in the results from the two groups: Deng et al. show that amino acid withdrawal increases while amino acid stimulation decreases RagA polyubiquitination. Lin et al., on the other hand, show that amino acid stimulation increases RagA polyubiquitination. How can amino acid stimulation both decrease and increase RagA polyubiquitination? In addition, it is curious that both RNF152-mediated (Deng et al., 2015) and Skp2-mediated (Jin et al., 2015) RagA polyubiquitination enhance binding between RagA and GATOR1, events that occur upon amino acid withdrawal and amino acid stimulation, respectively. While Jin et al. indeed demonstrated that amino acid stimulation enhances the RagA-GATOR1 interaction, Deng et al. did not show similar data with amino acid withdrawal. Lastly, how are polyubiquitin chains removed from RagA? Which deubiquitylases (DUBs) are responsible?

The finding that two different E3 ligases regulate RagA function suggests that polyubiquitination may represent an underappreciated posttranslational modification (PTM) for regulation of diverse mTORC1 pathway components. Indeed, TRAF6 was shown to mediate K63-linked polyubiquitination of mTOR to promote mTORC1 function (Linares et al., 2013). As ubiquitination regulates target protein function in myriad ways, this PTM may modulate the mTORC1 pathway by a broad array of mechanisms, an exciting prospect for future work.

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Gear Up in Circles

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Two studies published in this issue of *Molecular Cell* (Rybak-Wolf et al., 2015) and in the April issue of *Nature Neuroscience* (You et al., 2015) independently report the upregulated expression of back-spliced circular RNAs (circRNAs) in brains and suggest that they have a potential to regulate synaptic function.

Non-polyadenylated transcriptomic analyses with specific biochemical and bioinformatic strategies have uncovered widespread expression of circular RNAs from back-spliced exons (circRNAs), further expanding the fast growing families of enlisted noncoding RNAs (ncRNAs). Accumulated lines of evidence have revealed that the biogenesis of circRNAs is catalyzed by canonical spliceosomal machinery (Starke et al., 2015) and can be facilitated by both *cis*-elements (Zhang et al., 2014) and *trans*-factors (Ashwal-Fluss et al., 2014; Conn et al., 2015). Although expressed at a low level in general and having mostly unknown functions, some circRNAs were identified to be more abundant than their linear transcripts in different cell types (Salzman et al., 2013). Additionally, circRNAs are preferentially back-spliced from neural genes (Ashwal-Fluss et al., 2014) and upregulated in neural tissues in an age-dependent manner in *Drosophila* (Westholm et al., 2014), implying a potential role of circRNAs in fly brain function. However, a more complete analysis and functional characterization of circRNA expression in mammalian brain has been lacking. In this issue of *Molecular Cell* and in the April

issue of *Nature Neuroscience*, two papers by Rybak-Wolf et al. (2015) and You et al. (2015) report the identification of thousands of conserved circRNAs highly expressed in mammalian brain. Many such circRNAs are upregulated during neurogenesis and are more enriched in synaptic processes than their linear isoforms. These findings further highlight a potential role of brain circRNAs in the nervous system.

To assess brain-specific circRNA expression, Rybak-Wolf et al. (2015) examined 29 human/mouse RNA sequencing (RNA-seq) datasets from dissected brain tissues or neuronal-differentiated cell lines using both computational and biochemical approaches. Results showed that circRNAs are highly expressed in mammalian brain with an overall enrichment in the cerebellum and are generally induced during neuronal differentiation from genes with pivotal roles in neurons (Figure 1). Although many circRNAs are increased together with their linear isoforms during this process, strikingly, some circRNAs are dynamically expressed independent of their linear transcripts, implying a regulated expression of brain circRNAs. The authors also found that the expression and sequences of many brain-specific circRNAs tend to be conserved from human to mouse, and even to *Drosophila*. Similarly, by profiling circRNA expression from a set of mouse tissues and different developmental stages of mouse brain using the PacBio sequencing platform, an approach that generates rolling circle cDNA from circRNAs to reveal the circular structure at a single-nucleotide resolution, You et al. (2015) drew the parallel conclusion that circRNAs are highly enriched and developmentally regulated in the brain.

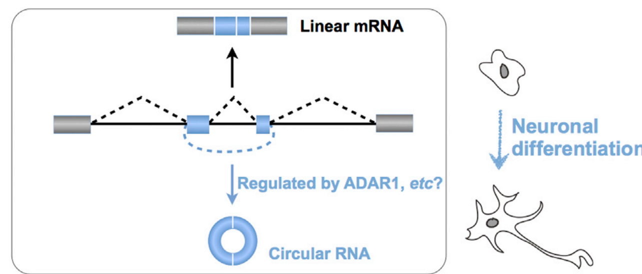

Figure 1. Upregulation of CircRNAs during Brain Development
A large group of circRNAs are highly expressed in mammalian brain and upregulated during neuronal differentiation (bottom) (Rybak-Wolf et al., 2015; You et al., 2015). Importantly, many of these circRNAs are dynamically expressed independent of their linear transcripts (top), implying regulated expression in the brain. Rybak-Wolf et al. (2015) further showed a negative correlation of editing level in flanking introns by ADAR1 with circRNA expression in neural tissues in flies and mammals, suggesting a negative role of ADAR1 on circRNA expression.

Importantly, both studies have uncovered that circRNAs are highly enriched in synapses by examining the abundance of circRNAs in purified synaptoneurosome fractions (Rybak-Wolf et al., 2015; You et al., 2015) and the microdissected synaptic neuropil from mouse hippocampus, a brain structure that exhibits robust synaptic plasticity (You et al., 2015). You et al. (2015) further confirmed this conclusion by utilizing high-resolution RNA in situ hybridization to show that some abundant circRNAs form specific particles in both neural cell bodies and dendrites. Surprisingly, the expression of a group of developmentally regulated circRNAs in brain is altered at the onset of synaptogenesis (You et al., 2015), indicating that some circRNAs may affect neuronal functions by an unknown mechanism. As some circRNAs are capable of binding miRNAs, and maybe RNA binding proteins (RBPs), it is possible that circRNAs might be involved in the informatic flow from neuron body to synapse by acting as carriers for miRNAs or RBPs. However, the current study with limited datasets suggests that these circRNAs do not likely serve as sponges for miRNA or RBPs (You et al., 2015). Thus, how circRNAs involve in synapse function remains to be further investigated.

The identification of brain/synapse-specific expression of circRNAs is interesting. What factors regulate the elevated expression of circRNAs in brain? A recent study has suggested a role of adenosine deaminase acting on RNA (ADAR) in the suppression of circRNA expression in *C. elegans* (Ivanov et al., 2015). Inverted repeated Alu pairs (IRAlus) offer an efficient way to promote circRNA formation in humans (Zhang et al., 2014). These RNA pairs are known to be catalyzed by ADAR to generate adenosine-to-inosine modifications, resulting in reduced RNA pairing and thus less-efficient back-splicing for circRNA formation. Rybak-Wolf et al. (2015) further showed a negative correlation between editing level in flanking introns by ADAR1 and circRNA expression in neural tissues in flies and mammals. However, the effect of ADAR1 editing activity on circRNA upregulation during neuronal differentiation could be limited, as exemplified by the observation that only a handful of cases were affected by ADAR1 knockdown (Rybak-Wolf et al., 2015). This could be due to the fact that the editability of Alu elements in introns is generally at a very low level and/or that ADAR1 might regulate circRNA biogenesis independent of its editing activity by directly acting as an RBP (Chen et al., 2015). In fact, RBPs such as Muscleblind (Ashwal-Fluss et al., 2014) and Quaking (Conn et al., 2015) were shown to bridge two flanking introns to induce back-splicing, resulting in upregulated circRNA formation.

Together, these two papers provide a valuable circRNA catalog in mammalian brain and shed new light on their potential function in the nervous system. Questions remain, including what other mechanisms account for the upregulated circRNAs in mammalian brain and what these brain-circRNAs do in the nervous system. As a large portion of circRNAs is upregulated along with their linear transcripts during neural induction (Rybak-Wolf et al., 2015), it is possible that the enhanced transcription elongation rate of brain-specific genes could result in the activation of back-splicing for circRNAs. Alternatively, the accumulation of very stable circRNAs may lead to their abundance in neuron, while their linear isoforms undergo more rapid turnover. Moreover, what other neuronal-specific RBPs also regulate circRNA biogenesis in brain? Since alternative splicing is prevalent in mammalian brain, is back-splicing co-regulated by similar mechanisms? Finally, as these studies have focused on the circRNA profiling in mammalian brain, it will be of great interest to annotate circRNAs in other systems, illustrated by the

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recent finding that hundreds of circRNAs are regulated during human epithelial-mesenchymal transition (Conn et al., 2015).

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