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The Ups and Downs of BDNF in Rett Syndrome

Rett syndrome (RTT) is an X-linked postnatal neurodevelopmental disorder, which is primarily caused by mutations in the gene encoding methyl-CpG binding protein 2 (MeCP2). A number of MeCP2 target genes have been identified, including the neurotrophic factor BDNF; however, the functional relevance of these targets has not been established. In this issue of *Neuron*, Chang et al. provide the first in vivo evidence for a functional interaction between BDNF and MeCP2.

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder. About 80% of the patients carry mutations in a gene located on the X chromosome, encoding a methyl-CpG binding protein, MeCP2 (Amir et al., 1999). This disorder is almost exclusively diagnosed in young females because males carrying similar mutations often die perinatally with severe congenital encephalopathy. After apparently normal development for 6–18 months, RTT patients show regression of head growth and loss of speech and acquired motor skills. Purposeful hand use is replaced by stereotypic hand-wringing movements. Patients display breathing irregularities, ataxia, seizures, scoliosis/kyphosis, autistic behavior, and severe mental retardation (Hagberg et al., 1983). Despite these symptoms, with proper care, RTT patients often survive into adulthood. Interestingly, after the initial crisis associated with the onset of the RTT symptoms, no further regression is apparent, suggesting that the condition does not involve progressive neurodegeneration.

Before the discovery of *Mecp2* as the primary disease gene for RTT, MeCP2 was known to bind to methylated DNA at methyl-CpG sites. MeCP2 binding translates DNA methylation into a gene-silencing signal by recruiting inactive chromatin remodeling complexes including histone deacetylases (HDACs) and histone H3 lysine 9 methyltransferases (H3K9-HMTs) (Fuks et al., 2003; Nan et al., 1998). MeCP2 contains two major functional domains, the methyl-CpG binding domain (MBD) and the transcriptional repression domain (TRD). A number of RTT mutations of MeCP2 have been found to cluster within either the MBD or TRD regions, which in most cases, render MeCP2 incapable of binding to methylated DNA and/or repressing gene transcription. Mouse knockout studies on MeCP2 further support the notion that loss-of-function mutations of *Mecp2* represent the primarily cause of RTT (Chen et al., 2001; Guy et al., 2001). However, more recent studies on the R168X (truncation) mutation of *Mecp2* as well as *Mecp2* overexpression transgenic mice indicated that enhanced *Mecp2* function or methyl-CpG binding affinity could also lead to neurological dysfunction related to RTT (Collins et al., 2004).

The attempts to reveal RTT etiology simply from loss of MeCP2 function in transcription repression have created some surprises. Efforts toward identification of genes repressed by MeCP2 by gene expression profiling yielded almost no significant results, which led to the conclusion that MeCP2 is not a global gene repressor (Tudor et al., 2002). One potential caveat of the approach taken is the heterogeneity of brain tissue, which contains many different types of neurons, glia, as well as cells of nonneural origin. It is likely that MeCP2 regulates different sets of genes in different cell types. Therefore using whole brain would significantly mask cell-type-specific changes in gene expression that result from MeCP2 deficiency. By using chromatin immunoprecipitation (ChIP) as well as gene expression analyses on *Mecp2*$^{+/y}$ and *Mecp2*$^{-/y}$ perinatal mouse cortical cultures that are less heterogeneous, the first mammalian neuronal target gene for MeCP2 was identified—brain-derived neurotrophic factor (*Bdnf*) (Chen et al., 2003; Martinowich et al., 2003). Subsequently, Dlx5 and several glucocorticoid-regulated genes were discovered as MeCP2 targets (Horike et al., 2005; Nuber et al., 2005). However, whether misregulation of these genes due to MeCP2 deficiency contributes, in any aspect, to RTT remains to be determined.

In this issue of *Neuron*, Chang et al. (2006) provide in vivo evidence for a functional interaction between BDNF and MeCP2. The story begins with a surprise, i.e., BDNF protein levels decrease in *Mecp2*$^{-/y}$ cortex, cerebellum, and the rest of the brain, respectively, by 21%, 41%, and 55% of wild-type levels. Because *Bdnf* is a target of repression by MeCP2, it is somewhat unexpected that BDNF levels decrease in the absence of MeCP2.

Three years ago, Chen et al. demonstrated that in neonatal *MeCP2*$^{-/y}$ mouse cortical cultures with neuronal activity blocked by TTX, there was an approximately 2-fold increase in the *Bdnf* exon IV(equivalent to rat Bndf exon III)-specific mRNA as compared to wild-type cultures (Chen et al., 2003). Given that these cortical cultures contain BDNF-expressing glutamatergic excitatory neurons, GABAergic-inhibitory neurons, which do not express BDNF even in the absence of MeCP2, and low levels cortical astrocytes/progenitors, this 2-fold increase likely underestimates the increase that would be observed from a population of pure resting excitatory neurons. In addition, because mouse *Bndf* can be transcribed from five different exon-specific promoters, it is unclear how this 2-fold increase in exon IV-specific mRNA affects the total mRNA and protein levels of BDNF. *Bdnf* is known to be robustly upregulated in response to neuronal activity. Thus, the reduction in overall cortical neuronal activity seen in *Mecp2*$^{-/y}$ mice is expected to affect *Bdnf* expression dramatically. This additional effect would override the increase in *Bdnf* basal transcription that results from lack of repression in resting cortical neurons because of MeCP2 deficiency (Figure 1).

It is worth mentioning that the reduction in *Bdnf* expression is not observed in 2 week old *Mecp2*$^{-/y}$ cortices in which decreased firing of layer V neurons is already apparent, suggesting that downregulation of BDNF is a later and indirect outcome of MeCP2 deficiency. Although it

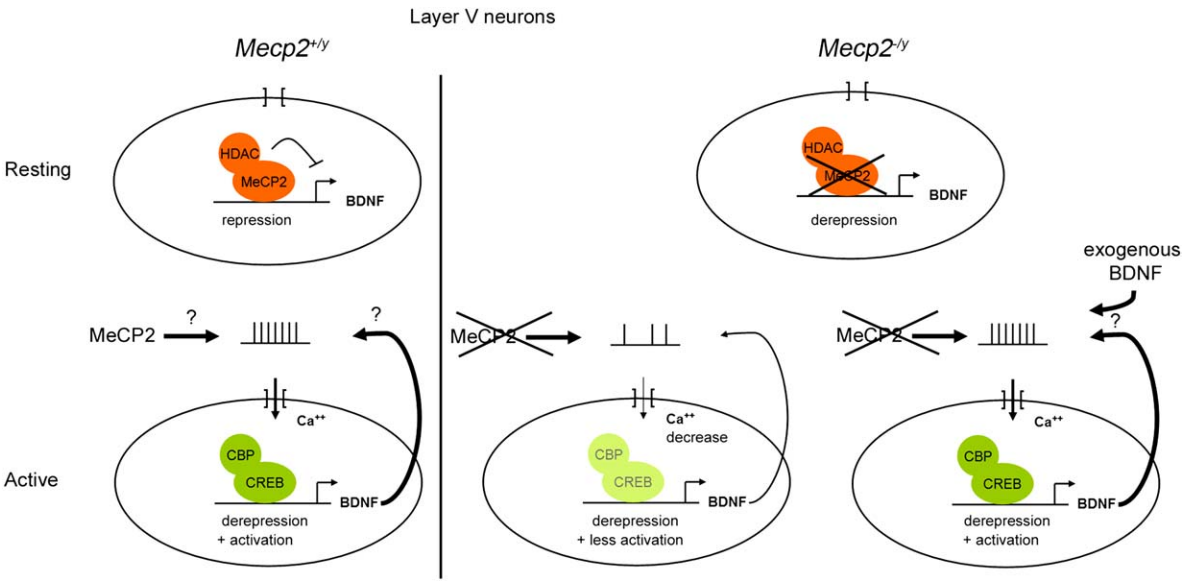

Figure 1. Cell-Autonomous and -Nonautonomous Functions of MeCP2 in *Bndf* Regulation and a Functional Interaction between MeCP2 and BDNF

In resting *Mecp2*$^{+/y}$ layer V neurons, BDNF exon IV promoter is repressed by the MeCP2/mSin3A/HDAC complex, which dissociates from the promoter after neuronal activity and is replaced by a transcriptional activation complex that includes CREB and CBP/p300. In resting *Mecp2*$^{-/y}$ neurons, the BDNF exon IV promoter is derepressed. However, without neuronal activity, no robust activation of the promoter can be detected. In *Mecp2*$^{-/y}$ cortices, neuronal activity decreases. Even though the promoter is derepressed because of MeCP2 deficiency, it can only be moderately activated because of decreased neuronal activity. In the case of BDNF overexpression, restored cortical neuronal activity potentially leads to a reversal of endogenous BDNF expression. It is currently unclear how MeCP2 and BDNF coordinately regulate neuronal activity.

wouldn't be surprising that the 30% decrease in the overall brain BDNF levels may contribute to the later disease progression of RTT, there is currently no evidence indicating that the direct and/or indirect regulation of BDNF by MeCP2 contributes to RTT etiology.

Elegant mouse genetic approaches enabled Chang et al. to convincingly demonstrate that forebrain-specific knockout of the *Bdnf* gene in *Mecp2*$^{-/y}$ mice exacerbated the RTT-like phenotypes. Remarkably, forebrain-specific overexpression of BDNF rescued a subset of RTT-like phenotypes in *Mecp2*$^{-/y}$ mice, including reduced lifespan, reduced motor activity, and reduced layer V neuronal firing frequency. These findings strongly suggest that there is a functional interaction between MeCP2 and BDNF and that brain BDNF levels can modulate RTT disease progression.

Chang et al. also report that the forebrain-specific knockout of *Bdnf* mimicked a subset of the phenotypes observed in the *Mecp2* knockout, including reduced brain weight, reduced olfactory and hippocampal neuronal sizes, and hindlimb clasping. However, overexpression of *Bdnf* has not been shown to effectively rescue these phenotypes shared by the two knockouts. In contrast, the phenotypes of *Mecp2*$^{-/y}$ mice that can be rescued by BDNF are not detectable in *Bdnf* knockout. Therefore, it is likely that BDNF has widespread neuromodulatory effects throughout the brain, some but not all of which overlap with MeCP2 function. A future mechanistic insight into the functional interaction between BDNF and MeCP2 will advance our understanding of RTT etiology.

Several new approaches and issues are currently emerging regarding RTT research: (1) cell-type-specific gene regulation by MeCP2 will soon be revealed by gene-expression studies with *Mecp2* loss- and gain-of-function transgenic mice together with neuronal subtype lineage labeling technologies; (2) genome-wide ChIP-on-chip technology with improved MeCP2 antibodies or epitope-tagged MeCP2 knockin mice, or cultured neuronal cells will help identify MeCP2 binding targets, which may or may not change expression upon *Mecp2* deletion; (3) more studies will be focused on posttranscriptional regulation of synaptic transmission by MeCP2; and (4) more attempts will be made to determine the role of the MeCP2 deficiency-induced imbalance between excitatory (glutamatergic) and inhibitory (GABAergic) circuitries in RTT. Finally, it has been shown that *Mecp2* null male mice (*Mecp2*$^{-/y}$) display symptoms similar to RTT female patients and female *Mecp2* heterozygote mice (*Mecp2*$^{+/-}$) display much more subtle neurological deficits, demonstrating a significant difference between mouse models and human patients. Therefore, human cell-culture models for RTT may contribute important complementary information to our understanding of the disease.

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Selected Reading

Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. (1999). Nat. Genet. *23*, 185–188.

Chang, Q., Khare, G., Dani, V., Nelson, S., and Jaenisch, R. (2006). Neuron *49*, this issue, 341–348.

Chen, R.Z., Akbarian, S., Tudor, M., and Jaenisch, R. (2001). Nat. Genet. *27*, 327–331.

Chen, W.G., Chang, Q., Lin, Y., Meissner, A., West, A.E., Griffith, E.C., Jaenisch, R., and Greenberg, M.E. (2003). Science *302*, 885–889.

Collins, A.L., Levenson, J.M., Vilaythong, A.P., Richman, R., Armstrong, D.L., Noebels, J.L., David Sweatt, J., and Zoghbi, H.Y. (2004). Hum. Mol. Genet. *13*, 2679–2689.

Fuks, F., Hurd, P.J., Deplus, R., and Kouzarides, T. (2003). Nucleic Acids Res. *31*, 2305–2312.

Guy, J., Hendrich, B., Holmes, M., Martin, J.E., and Bird, A. (2001). Nat. Genet. *27*, 322–326.

Hagberg, B., Aicardi, J., Dias, K., and Ramos, O. (1983). Ann. Neurol. *14*, 471–479.

Horike, S., Cai, S., Miyano, M., Cheng, J.F., and Kohwi-Shigematsu, T. (2005). Nat. Genet. *37*, 31–40.

Martinowich, K., Hattori, D., Wu, H., Fouse, S., He, F., Hu, Y., Fan, G., and Sun, Y.E. (2003). Science *302*, 890–893.

Nan, X., Ng, H.H., Johnson, C.A., Laherty, C.D., Turner, B.M., Eisenman, R.N., and Bird, A. (1998). Nature *393*, 386–389.

Nuber, U.A., Kriaucionis, S., Roloff, T.C., Guy, J., Selfridge, J., Steinhoff, C., Schulz, R., Lipkowitz, B., Ropers, H.H., Holmes, M.C., and Bird, A. (2005). Hum. Mol. Genet. *14*, 2247–2256.

Tudor, M., Akbarian, S., Chen, R.Z., and Jaenisch, R. (2002). Proc. Natl. Acad. Sci. USA *99*, 15536–15541.

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Think Globally, Act Locally: Local Translation and Synapse Formation in Cultured *Aplysia* Neurons

Synapse formation is initiated by cell-cell contact between appropriate pre- and postsynaptic cells and is followed by recruitment of protein complexes in both pre- and postsynaptic compartments. In this issue of *Neuron*, Lyles et al. show that in cultured *Aplysia* neurons, clustering of an mRNA at nascent synapses is not only induced by the recognition between synaptic partners, but is also required for further synaptic development and maintenance.

Neurons are large, highly differentiated cells with complex morphologies. Synapses, the business ends of neurons, are frequently localized in distal neurites, far away from the cell body. How do distal neurites acquire and maintain the repertoire of proteins that are required for synaptic function? In many cases, passive diffusion is not fast enough to efficiently deliver proteins and organelles to synapses due to the great distance between the neuronal cell body and distal neurites. Two active mechanisms have been proposed to supply the distal compartments with their constituents. First, protein may be synthesized in the cell body and transported to distal neurites. A large family of molecular motors traffic cytosolic components and organelles back and forth between neurites and the cell body (Vale, 2003). Alternatively, protein synthesis may take place locally in neurites. A growing body of evidence supports the notion that local translation in neurites is important for the development and plasticity of neural circuits. It is worth noting that local translation requires the presence of mRNA, ribosomes, and translational machinery at neurites, which is likely to depend on molecular motor-based intracellular trafficking.

Classic experiments performed by Oswald Steward and colleagues showed that polyribosomes were selectively localized beneath postsynaptic sites in the dendrites of CNS neurons (Steward, 1983; Steward and Fass, 1983; Steward and Levy, 1982). Later studies showed that both mRNA and translation machinery were present at dendritic spines (reviewed by Steward and Schuman, 2001). The presence of particular transcripts in axonal growth cones and their importance in axon guidance have also been suggested (Brittis et al., 2002; Campbell and Holt, 2001). Still, many questions regarding mRNA localization and function at synapses have not been clearly addressed. For example, at what point in time does a particular mRNA localize to synapses during the course of synapse formation and maturation? What developmental events trigger this clustering at synapses? Is the increased concentration of mRNA at synapses due to redistribution of preexisting mRNA or due to new transcription? Finally, is synaptic mRNA required for synapse formation? In this study, Lyles et al. (2006) presented an elegant set of experiments to address these questions while studying the neuropeptide sensorin.

In culture, *Aplysia* neurons form functional synapses, whose activity can be readily measured with electrophysiology. Remarkably, synapse formation in vitro maintains target specificity found in intact animals, whereby isolated *Aplysia* sensory neurons preferentially form synapses onto the motor neurons that are their natural postsynaptic partners. These features, combined with the ability to perform RNA interference and in situ hybridization experiments, make cultured *Aplysia* neurons an ideal system for testing mRNA localization and function at synapses. The authors found sensorin transcript in a cDNA library from isolated processes of sensory neurons. Consistent with previous reports, they found that sensorin mRNA is localized in distal neurites (Brunet et al., 1991). More specifically, they reported that sensorin mRNA is particularly concentrated at presynaptic sites. Furthermore, this clustering effect is most efficiently induced when the appropriate target neurons are cocultured, suggesting that recognition between synaptic partners triggers mRNA localization at synapses.

Next, the authors analyzed the mechanism of sensorin mRNA localization by asking whether the clustering of mRNA is due to redistribution of existing mRNA or synthesis of new mRNA. Surprisingly, they found that a transcriptional inhibitor, actinomycin D, blocks synaptic accumulation of sensorin mRNA. This result implies that the synaptically localized sensorin mRNA is a new population of mRNA that is induced by synapse formation signals. It also suggests that there must be differences between the newly synthesized mRNA and the preexisting mRNA to aid the selective accumulation of new mRNA at synapses. Therefore, signals generated by nascent synapses must travel to the nucleus to stimulate the transcription of sensorin. The newly synthesized