Brain-Derived Neurotrophic Factor and Rett Syndrome

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Abstract

Rett syndrome (RTT) is a devastating neurodevelopmental disorder with autistic features caused by loss-of-function mutations in the gene encoding methyl-CpG-binding protein 2 (*MECP2*), a transcriptional regulatory protein. RTT has attracted widespread attention not only because of the urgent need for treatments, but also because it has become a window into basic mechanisms underlying epigenetic regulation of neuronal genes, including BDNF. In addition, work in mouse models of the disease has demonstrated the possibility of symptom reversal upon restoration of normal gene function. This latter finding has resulted in a paradigm shift in RTT research and, indeed, in the field of neurodevelopmental disorders as a whole, and spurred the search for potential therapies for RTT and related syndromes. In this context, the discovery that expression of BDNF is dysregulated in RTT and mouse models of the disease has taken on particular importance. This chapter reviews the still evolving story of how MeCP2 might regulate expression of BDNF, the functional consequences of BDNF deficits in *Mecp2* mutant mice, and progress in developing BDNF-targeted therapies for the treatment of RTT.

Keywords

BDNF • MeCP2 • TrkB • Autism spectrum disorders • Neurodevelopmental • Brainstem

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Abbreviations

The following abbreviations are used for the gene encoding methyl-CpG-binding protein 2 and its protein product:

MECP2, BDNF	Human gene
Mecp2, Bdnf	Mouse gene
MeCP2, BDNF	Protein

1 Introduction

Rett syndrome (RTT) is a complex neurodevelopmental disorder that affects approximately 1 in 10,000 live female births worldwide (Chahrour and Zoghbi 2007). RTT is characterized by apparently normal early postnatal development with neurological symptoms appearing around 6–18 months of age. The subsequent course of the disorder is variable and patients exhibit a diverse array of symptoms that generally includes loss of acquired speech, head growth deceleration, autistic features such as emotional withdrawal and diminished eye contact, motor stereotypies, early hypotonia followed by rigidity, epileptiform seizures, exaggerated responses to stress, and severe respiratory and autonomic (cardiac and gastrointestinal) dysfunction (Chahrour and Zoghbi 2007; Hagberg et al. 1983; Katz et al. 2009; Shahbazian and Zoghbi 2002; Vorsanova et al. 2004; Weese-Mayer et al. 2006, 2008). Up to a quarter of RTT patients may die prematurely of cardiorespiratory failure (Kerr et al. 1997).

The vast majority of typical RTT cases result from loss-of-function mutations in the gene encoding methyl-CpG-binding protein 2 (MeCP2; Amir et al. 1999; Chahrour and Zoghbi 2007), a transcriptional regulatory protein (Klose and Bird 2006). Over 250 different *MECP2* mutations have been identified in RTT patients, most of which tend to cluster either within the methyl-binding or transcription repression domains. The *MECP2* gene is X-linked, and affected females are heterozygotes and somatic mosaics for MeCP2, i.e., cells in which the mutated allele occurs on the inactive X are phenotypically normal for MeCP2 expression, whereas cells in which the mutated allele occurs on the active X are mutant. Disease phenotype is therefore affected not only by the specific *MECP2* mutation but also by the skewing of X chromosome inactivation; individuals in which inactivation is skewed towards the mutant allele are less severely affected, and vice versa. Hemizygosity in males is usually fatal, and the chances of homozygosity in females are exceedingly small, given that most disease-causing mutations arise in the paternal germ line and child-bearing by affected females is extremely rare.

The full scope of MeCP2 function in neurons remains a subject of some controversy. Although it is clear that MeCP2 binds methylated DNA and can potently silence transcription (Klose and Bird 2006), additional functions, including

transcriptional activation (Chahrour et al. 2008), regulation of RNA processing (Young et al. 2005), and control of higher order chromatin structure (Georgel et al. 2003), have been proposed. Moreover, it is unclear whether or not MeCP2 selectively regulates transcription of specific genes or, alternatively, acts globally to regulate chromatin state across the genome. A recent study by Skene et al. (2010) demonstrated that MeCP2 protein is abundantly expressed in neurons at levels comparable to histone octamers, i.e., sufficient to blanket the genome at methylated CpG dinucleotides. Therefore, these authors have suggested that the primary function of MeCP2 is to globally repress spurious transcription, e.g., of nucleotide repeats across the genome rather than to dynamically regulate expression of specific genes. However, Skene et al. (2010) showed that, in addition to its widespread binding across genome, MeCP2 also shows peaks of even higher binding at specific sites within promoter regions. Whether or not this is evidence for a more specific role in dynamic regulation of particular genes remains unclear. Nonetheless, what is clear is that expression of many genes is disrupted, either directly or indirectly, by loss-of-function mutations in *MECP2* and that the complexity of RTT is related to the diversity of affected gene targets.

2 Regulation of BDNF Expression, Trafficking, and Secretion by MeCP2

The debate concerning the role of MeCP2 in gene regulation is particularly relevant to understanding the evolution of current thinking regarding BDNF and the pathogenesis of RTT. The initial suggestion that dysregulation of BDNF expression might play a role in RTT came from in vitro evidence that BDNF is a transcriptional target of MeCP2 and repressed by MeCP2 binding to BDNF promoter regions. Specifically, Chen et al. (2003) and Martinowich et al. (2003) used chromatin immunoprecipitation to demonstrate binding of MeCP2 protein to BDNF promoter IV (referred to at the time as promoter III), one of nine BDNF promoters and one that is particularly important for activity-dependent regulation of BDNF expression. Moreover, MeCP2 binding appears to recruit a transcriptional repressor complex that includes HDAC1 and Sin3A to the BDNF locus (Martinowich et al. 2003). Chen et al. (2003) and Martinowich et al. (2003) further showed that MeCP2 binding to the BDNF gene was dynamic and subject to regulation in cultured neurons by exposure to depolarizing stimuli, such as elevated potassium chloride (KCl). Specifically, strong chemical depolarization reduces MeCP2 binding to BDNF promoter IV (Martinowich et al. 2003) in association with a change in the phosphorylation state of MeCP2 (Chen et al. 2003), reduces methylation of promoter IV (Martinowich et al. 2003), and increases BDNF expression (see also Ballas et al. 2005). Subsequently, Zhou and colleagues (Zhou et al. 2006) demonstrated that phosphorylation of MeCP2 at serine 421 is particularly important for activity-dependent increases in BDNF expression in cultured hippocampal neurons. Consistent with this repression model, Chen et al. (2003) showed that *Mecp2* null embryonic cortical neurons cultured in the presence of blockers of

neuronal activity exhibited higher levels of BDNF exon IV mRNA than wild-type cells. However, in the presence of a depolarizing concentration of KCl, wild-type and mutant cells exhibited similar levels of BDNF expression, which the authors interpreted as consistent with BDNF already being derepressed in the mutant cells to levels similar to those achieved in wild-type cells upon stimulation. More recently, evidence has emerged that BDNF expression can also be regulated by the acetylation state of MeCP2 in a manner consistent with the repression model. Specifically, mice lacking functional SIRT1, a nicotinamide-adenine dinucleotide-dependent histone deacetylase, exhibit increased MeCP2 binding to the BDNF exon IV promoter and decreased levels of BDNF mRNA and protein (Zocchi and Sassone-Corsi 2012).

The hypothesis that MeCP2 normally represses BDNF transcription predicted that loss of MeCP2 function in RTT, or mouse models of the disease, would be associated with elevated BDNF expression. However, this prediction has not been borne out, as *Mecp2* null or heterozygous mice exhibit *reduced* levels of BDNF mRNA and protein in vivo (Chang et al. 2006; Ogier et al. 2007; Wang et al. 2006). Similarly, two studies of postmortem brain samples from RTT patients have demonstrated reduced levels of BDNF mRNA (Abuhatzira et al. 2007; Deng et al. 2007). The BDNF mRNA and protein deficits observed in the brain and peripheral nervous system of *Mecp2* mutant mice are progressive (Chang et al. 2006; Ogier et al. 2007; Wang et al. 2006), being virtually undetectable at birth and declining to as much as 50 % of wild-type levels in some tissues by 5 weeks of age in male nulls (Wang et al. 2006). Moreover, the postnatal decline in BDNF levels occurs with a slower time course in heterozygous females compared to male nulls (Schmid et al. 2012). Clearly, these in vivo data are inconsistent with a model in which MeCP2 simply represses expression of BDNF.

Various hypotheses have been offered to explain how loss of MeCP2 function could lead to deficits in BDNF expression. One idea, already introduced above, is that MeCP2 activates rather than represses gene expression. In support of this hypothesis, Chahrour et al. (2008) showed that global overexpression of MeCP2 in mice is associated with increased expression of BDNF mRNA in the hypothalamus, whereas MeCP2 loss is associated with decreased BDNF. Similarly, selective deletion of *Mecp2* from Sim-1-positive neurons also causes a reduction in BDNF in the hypothalamus (Fyffe et al. 2008). The activator hypothesis is also supported by a recent report by Li et al. (2013) demonstrating global reductions in transcription and Akt/mTOR-dependent protein translation—including BDNF—in human iPSC-derived neurons in which the *Mecp2* gene was deleted using TALEN-mediated DNA editing. One caveat to these findings is that the possible contribution of decreased BDNF mRNA and/or protein stability, rather than decreased gene transcription per se, has not been ruled out. Further support for the activator model comes from studies showing that derepression of microRNA (miRNA)-mediated inhibition of MeCP2 translation in cultured neurons increases expression not only of MeCP2 but BDNF as well (Klein et al. 2007).

A recent approach to resolving the repressor *versus* activator debate is the "dual operation model" (Li and Pozzo-Miller 2013). This model is motivated by data

from one study showing that either knockdown or overexpression of MeCP2 in cultured neurons leads to increased expression of BDNF (Larimore et al. 2009), as well as evidence that MeCP2 can undergo diverse posttranslational modifications, including phosphorylation, acetylation, and ubiquitylation, leading to unique associations with either co-repressors or co-activators (Gonzales et al. 2012).

A second hypothesis that has been proposed to explain decreased BDNF expression in the absence of MeCP2 function is that MeCP2 normally represses the activity of repressors of BDNF expression, i.e., the RE1 silencing transcription factor (REST)/CoREST complex (Abuhatzira et al. 2007). This model is based on data from mice and humans demonstrating elevated levels of REST/CoREST in RTT patients and *Mecp2*-deficient mice, presumably leading to reduced BDNF expression through repressive interactions with the RE1 element in BDNF promoter regions. A third hypothesis is that reduced BDNF expression in *Mecp2* null neurons is a consequence of reduced neuronal activity (Sun and Wu 2006). This idea was based on the finding that cortical neurons from *Mecp2* null mice exhibit reduced firing rates associated with a loss of excitatory synaptic drive (Dani et al. 2005). However, we found that even after exposure to strongly depolarizing stimuli in vitro, *Mecp2* null cells express less BDNF protein than wild-type, indicating that differences in activity alone are unlikely to account for BDNF deficits in the absence of MeCP2 (Ogier et al. 2007). Thus, at present, the normal role of MeCP2 in regulating BDNF expression, as well as the mechanism (s) responsible for reduced BDNF levels in the RTT brain, remain to be clarified. One possibility is that, although loss of MeCP2 may result in derepression of BDNF gene expression, translation and/or stability of the protein may also be adversely affected, resulting in a net decrease in BDNF levels in the RTT brain. In support of this possibility, Wu et al. (2010) recently demonstrated that MeCP2 controls transcription of several microRNAs (miRNAs) that target the 3′ UTR of *Bdnf* mRNA, some of which are upregulated in the absence of MeCP2 function and negatively regulate *Bdnf* mRNA levels. Conversely, inhibition of two such miRNAs, miR-381 and miR-495, in both wild-type and *Mecp2* null neurons in vitro, increased levels of *Bdnf* mRNA and BDNF protein. Thus, Wu et al. (2010) proposed that, in the absence of MeCP2 function, the net effect of direct derepression of *Bdnf* mRNA, combined with depression of miRNAs that negatively regulate *Bdnf* mRNA, is reduced BDNF levels. This hypothesis requires further testing, as Wu et al. (2010) also identified miRNAs that target *Bdnf* mRNA and are downregulated in the absence of MeCP2 function. In particular, it will be critical to define the stoichiometry of these positive and negative influences on *Bdnf* transcription, translation, and stability in vivo in order to fully understand the role of miRNAs in BDNF protein deficits in RTT.

In addition to dysregulation of BDNF expression, loss of MeCP2 also appears to disrupt regulated secretion and transport of BDNF. Although mature sensory neurons lacking MeCP2 express lower levels of BDNF protein, they actually secrete a larger proportion of their total BDNF content than wild-type cells, at least in cell culture (Ladas et al. 2009). However, this enhanced secretion is not sufficient to completely compensate for reduced levels of BDNF expression, and

the absolute amount of BDNF released by mutant cells is nonetheless lower than wild-type. This is also seen at mossy fiber inputs onto CA3 pyramidal neurons in *Mecp2* null mice, in which activity-dependent BDNF release is reduced compared to wild type, resulting in reduced activation of TrkB and reduced signaling through TRPC3 channels (Li et al. 2012). On the other hand, in newborn *Mecp2* null neurons, which do not yet exhibit a significant deficit in BDNF expression, the absolute amount of BDNF released is actually greater than wild-type (Wang et al. 2006). These data raise the possibility that during early development, enhanced secretion of BDNF from *Mecp2* null cells could derange developmental processes that depend on tight coupling between neuronal activity and BDNF release, such as activity-dependent refinement of synaptic connections (Lein and Shatz 2000). Enhanced BDNF release appears to be just one manifestation of a more widespread dense core vesicle phenotype in *Mecp2* null mice. Studies of catecholamine release in *Mecp2* null adrenal chromaffin cells demonstrated that the readily releasable pool of dense core vesicles is significantly larger and individual vesicles are more fusigenic than in wild-type cells, resulting in hypersecretion of epinephrine (Ladas et al. 2009; Wang et al. 2006). Given that BDNF is also a dense core vesicle cargo (Decker et al. 2010; Farhadi et al. 2000; Luo et al. 2001; Salio et al. 2007; Wu et al. 2004), similar mechanisms may underlie the BDNF secretory phenotype in *Mecp2* null mice.

Recent studies indicate that BDNF signaling in *Mecp2* mutants is also impacted by deficits in axonal transport, resulting from dysregulation of huntingtin (Htt)- and huntingtin-associated protein (Hap1)-dependent vesicle trafficking (Roux et al. 2012). Specifically, the velocity of vesicular BDNF transport in cortico-striatal projection neurons is impaired by loss of MeCP2. Given the importance of cortically derived BDNF for the maintenance of striatal medium-spiny neurons (Baquet et al. 2004), these data raise the possibility that deficits in BDNF transport from the cortex contribute to striatal pathology in RTT (cf., Stearns et al. 2007).

3 Topography of BDNF Deficits in Mouse Models of RTT

The time course and distribution of BDNF deficits resulting from loss of MeCP2 have been studied in some detail in *Mecp2* null and heterozygous mice (Chang et al. 2006; Ogier et al. 2007; Wang et al. 2006; Deogracias et al. 2012). The earliest and most dramatic deficits in BDNF mRNA and protein occur in the vagal sensory nodose ganglion (NG), followed by the brainstem, cerebellum, and cortex (Chang et al. 2006; Ogier et al. 2007; Wang et al. 2006). In NG sensory neurons, for example, BDNF mRNA and protein levels fall to approximately 50 % wild-type values within 5 weeks after birth (Ogier et al. 2007), leading to synaptic dysfunction in vagal afferent inputs to the brainstem (see below). Within the brain, the effect of MeCP2 loss on BDNF levels is not uniform across cell groups. For example, although *Mecp2* null mutants exhibit marked decreases in BDNF immunostaining in the neuropil of some brainstem nuclei, such as the nucleus tractus solitarius (nTS), nucleus ambiguus, and nucleus locus coeruleus (LC), others, such as the gracile and principal sensory

trigeminal nuclei, are only mildly affected or unchanged (Kline et al. 2010). Mechanisms that underlie the differential temporal and spatial patterns of BDNF decline in the *Mecp2* mutant brain have not been defined. Recent data indicate that regional BDNF deficits in *Mecp2* null mutants are accompanied by reduced levels of TrkB phosphorylation without a change in total TrkB expression (Schmid et al. 2012).

4 BDNF Deficits in Mouse Models of RTT: Functional Consequences

With a few exceptions, relatively little is known about the specific functional consequences of reduced BDNF expression in *Mecp2* mutants and RTT patients. Morphologic and synaptic phenotypes observed in the brains of RTT patients and/or *Mecp2* null mutants, including decreased brain weight and neuronal size, reduced dendritic arborizations and impaired hippocampal long-term potentiation (reviewed in Chahrour and Zoghbi 2007), overlap with deficits seen in *Bdnf* loss-of-function mutants (Chang et al. 2006; Huang and Reichardt 2001). In addition, at least some of the behavioral features of *Mecp2* mutant mice, including irregular breathing and impaired locomotion, overlap to some degree with deficits observed in *Bdnf* mutants (Conover et al. 1995; Erickson et al. 1996). Moreover, genetic overexpression of BDNF in *Mecp2* null mutants can improve survival and locomotor function, whereas BDNF deletion hastens the onset of symptoms (Chang et al. 2006). However, few studies have examined how reduced BDNF availability in identified neural circuits is linked to specific functional deficits in RTT. What is clear is that because BDNF declines postnatally in *Mecp2* mutants, the size of neuronal populations that depend on BDNF for survival before birth is unaffected (Wang et al. 2006). Therefore, increasing attention has focused on the role of BDNF deficits in the maturation and function of the RTT brain after birth.

4.1 MeCP2 and Stimulation of Dendritic Growth by BDNF

MeCP2 plays a key role in mediating the effects of environmental stimuli, such as neuronal depolarization, on expression of genes required for neuronal maturation, including *BDNF* (Cohen et al. 2011; Ebert et al. 2013). For example, Zhou et al. (2006) demonstrated that phosphorylation of MeCP2 at serine 421 (ser421) is required for activity-dependent expression of BDNF in postnatal hippocampal neurons. BDNF, in turn, can stimulate ser421 phosphorylation of MeCP2, indicating that BDNF functions both upstream and downstream of MeCP2. MeCP2 phosphorylation at ser421 is also required for expression of mature dendritic morphologies in hippocampal neurons (Chapleau et al. 2009; Zhou et al. 2006), possibly by activating this BDNF signaling loop. In support of this possibility, overexpression of BDNF can reverse dendritic atrophy in hippocampal neurons that are null for *Mecp2* (Larimore et al. 2009).

4.2 BDNF and Synaptic Dysfunction in RTT

The potential synaptic consequences of BDNF loss have been studied in detail at primary afferent synapses between NG primary sensory neurons and second order neurons in the nTS. These synapses are the first site at which peripheral visceral sensory inputs impinge on central autonomic reflex pathways and thereby play a critical role in autonomic functions disrupted in RTT, such as respiratory, cardiovascular, and gastrointestinal homeostasis. Normally, BDNF plays a sensory gating function at these synapses by modulating postsynaptic responses to glutamate, the primary excitatory transmitter of visceral afferent neurons (Balkowiec et al. 2000). We hypothesized, therefore, that in *Mecp2* null mice, decreased BDNF expression in NG sensory neurons would be associated with a deficit in modulation of fast glutamatergic transmission at primary afferent synapses in nTS. Indeed, the amplitudes of spontaneous miniature and evoked EPSCs in nTS neurons are significantly increased in *Mecp2* null mice (Kline et al. 2010; Kron et al. 2012a), and accordingly, mutant cells are more likely than wild-type to fire action potentials in response to primary afferent stimulation (Kline et al. 2010). These changes occur without any increase in intrinsic neuronal excitability and are unaffected by blockade of inhibitory GABA currents. A prediction of these results is that autonomic reflexes mediated by primary afferent inputs to nTS would be disinhibited in the absence of MeCP2 function. This prediction has been borne out by studies demonstrating that the hypoxic ventilatory response, a reflex mediated by primary chemoafferent inputs to nTS, is markedly exaggerated in *Mecp2* null mice compared to wild-type controls (Bissonnette and Knopp 2006; Roux et al. 2008; Voituron et al. 2009). Similarly, *Mecp2* nulls exhibit a loss of habituation in the Breuer–Hering reflex, an nTS-mediated behavior that plays an essential role in regulating the post-inspiratory phase of the respiratory cycle (Stettner et al. 2007). More generally, these findings suggest that reduced sensory gating in nTS contributes to cardiorespiratory instability in RTT and that nTS is a site at which restoration of normal BDNF signaling could help to reestablish normal homeostatic controls. Indeed, exaggerated synaptic responses to primary afferent input in nTS are reversed by application of exogenous BDNF to brainstem slices in vitro (Kline et al. 2010). Moreover, respiratory function in vivo is improved by treatments that enhance BDNF/TrkB signaling in *Mecp2* mutants (see below).

4.3 BDNF and Hypothalamic Dysfunction in RTT

Feeding behavior and energy homeostasis are strongly influenced by BDNF/TrkB signaling in the hypothalamus (Noble et al. 2011; Rios et al. 2001). Specifically, increased levels of BDNF are associated with cessation of feeding and increased energy expenditure. Although the specific circuitry underlying the role of BDNF in feeding has not been completely defined, BDNF has been identified as a downstream effector of melanocortin-4 receptor (MC4R) signaling in the ventromedial hypothalamus (Noble et al. 2011; Xu et al. 2003), a key site for regulating feeding

and satiety. Fyffe et al. (2008) demonstrated that loss of *Mecp2* by Cre-mediated deletion specifically within Sim-1 expressing neurons in the hypothalamus results in reduced BDNF levels in *Mecp2* null neurons in the paraventricular nucleus, also a site of MC4R expression (Nicholson et al. 2007), as well as hyperphagia and obesity. Although the relevance of the obesity phenotype to RTT is unclear, these data provide further evidence that MeCP2 is required for maintaining normal levels of BDNF expression and metabolic homeostasis.

5 BDNF-Targeted Therapies for RTT

Recent studies in conditional *Mecp2* null mice have demonstrated that reactivation of the *Mecp2* gene, even in severely symptomatic animals, can rescue neurologic function to a remarkable degree (Guy et al. 2007). These findings indicate that deficits caused by loss of MeCP2 function are not due to irreversible changes in brain structure or function. In addition, as noted above, genetic overexpression of the BDNF gene in *Mecp2* null mice improves somatomotor function and prolongs life span (Chang et al. 2006), and exogenous BDNF can reverse synaptic deficits caused by MeCP2 deficiency (Kline et al. 2010). Together, these findings raise the possibility of rescuing neurologic function in *Mecp2* null mice and, eventually, RTT patients, by pharmacologic therapies that enhance BDNF/TrkB signaling. BDNF itself does not have good drug-like characteristics, i.e., limited half-life and poor blood–brain barrier penetration, thus motivating the search for alternative approaches to increasing BDNF/TrkB signaling in RTT. As discussed below, these approaches include enhancing expression of endogenous BDNF, increasing BDNF trafficking, and directly activating the TrkB receptor.

5.1 Increasing Expression or Delivery of Endogenous BDNF

In the first test of a BDNF-targeted therapeutic strategy, Ogier et al. (2007) examined whether or not pharmacologic elevation of endogenous BDNF expression with ampakine drugs could improve respiratory function in *Mecp2* null mice. Ampakines are benzamide derivatives that facilitate the activity of glutamatergic AMPA receptors and thereby increase expression of activity-dependent genes, including BDNF (Lynch and Gall 2006). Repeated administration of ampakines in rats and mice increases expression of BDNF mRNA and protein in the forebrain for several days (Lauterborn et al. 2003; Rex et al. 2006) and augments BDNF-dependent synaptic function (Ingvar et al. 1997; Porrino et al. 2005; Rex et al. 2006). Indeed, treatment of *Mecp2* null mutants with the ampakine CX546 for 3 days significantly increases BDNF levels in NG sensory neurons and reverses the respiratory tachypnea that is a prominent feature of breathing dysfunction in RTT (Ogier et al. 2007). Although additional studies are required to elucidate the mechanism of ampakine action in this model, these data are consistent with the hypothesis that BDNF deficits contribute to the respiratory phenotype of *Mecp2* null mice and that

BDNF signaling may be a pharmacological target for improving respiratory function in RTT. More recently, Deogracias et al. (2012) showed that fingolimod, a sphingosine-1 phosphate receptor agonist used to treat multiple sclerosis, increases BDNF in cultured neurons and protects against NMDA-induced neuronal death in a BDNF-dependent manner. In vivo, treatment of *Mecp2* mutant mice partially reversed BDNF deficits and also increased striatal volume, an index of BDNF signaling. Treated mice also showed improvement in locomotor behavior, a clinically relevant outcome measure for RTT patients. Finally, it is well known that BDNF expression in the rodent forebrain can be increased by environmental enrichment and exercise (cf., Cotman and Berchtold 2002). Indeed, rearing *Mecp2* mutant mice in an enriched environment, particularly at early stages of postnatal development, leads to improvements in motor and spatial learning, coordination, and anxiety, as well as hippocampal circuit function, that correlate well with increases in BDNF expression (Kondo et al. 2008; Lonetti et al. 2010).

Another potential strategy for enhancing BDNF/TrkB signaling in RTT is to increase the bioavailability of endogenous BDNF by promoting increased axonal transport and/or secretion. Recently, Roux et al. (2012) showed that cysteamine, a drug that increases vesicular trafficking of BDNF (Borrell-Pages et al. 2006), extends life span and improves motor function in *Mecp2* mutant mice.

5.2 Targeting the BDNF Receptor, TrkB

One potential limitation of pharmacologic approaches that globally increase BDNF is that BDNF activates receptors other than TrkB, including p75. The properties of BDNF binding to p75 as well as functioning as a full agonist at TrkB could lead to unwanted pleiotropic effects of elevated BDNF levels. An alternative approach is to directly activate TrkB; potential strategies include TrkB activating antibodies (Qian et al. 2006) and small molecules that function as direct TrkB ligands (Jang et al. 2010; Massa et al. 2010; Xie and Longo 2000). Our laboratory has recently examined the ability of a small molecule, non-peptide BDNF loop 2 domain mimetic, LM22A-4, which functions as a direct and specific partial agonist of TrkB, but not p75 (Massa et al. 2010), to increase TrkB activation and improve breathing in *Mecp2* mutant mice. LM22A-4 was developed by Longo, Massa, and colleagues by in silico screening for mimetics of BDNF loop domains that selectively activate TrkB and downstream signaling partners in vitro and in vivo (Han et al. 2012; Massa et al. 2010; Schmid et al. 2012). Recent studies in our laboratory have shown that LM22A-4 (1) reduces synaptic hyperexcitability in the brainstem respiratory network in brain slice preparations (Kron et al. 2012b), (2) reverses deficits in TrkB activation in the brainstem (Schmid et al. 2012), and (3) significantly improves respiratory function (Schmid et al. 2012), including the elimination of apneic breathing (Kron et al. 2012b), following systemic administration to symptomatic *Mecp2* null and heterozygous mice. Together, these data provide direct evidence linking TrkB signaling to respiratory dysfunction in mouse models

of RTT and further highlight the therapeutic potential of strategies aimed at enhancing BDNF/TrkB signaling for the treatment of RTT patients.

6 Summary

BDNF is only one of many genes whose expression is dysregulated in RTT (Chahrour et al. 2008). Nonetheless, given the multiplicity of roles played by BDNF signaling in brain maturation and neural circuit function across the life span, it is not surprising that deficits in BDNF protein levels have now been linked, either directly or indirectly, to diverse neurologic deficits in RTT, including reduced dendritic growth, breathing dysfunction, and impaired locomotion. Certainly, much more work is required to understand how BDNF deficits may contribute to the expression of specific RTT endophenotypes. It is encouraging, however, that the possibility of treating RTT using BDNF/TrkB-targeted therapies has already been established in principle in mouse models of the disease.

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