Mechanisms and therapeutic challenges in autism spectrum disorders: insights from Rett syndrome

Jorge Castro, Nikolaos Mellios, and Mriganka Sur

Purpose of review
A major challenge for understanding the neurodevelopmental disorders, including autism spectrum disorders (ASDs), is to advance the findings from gene discovery to an exposition of neurobiological mechanisms that underlie these disorders and subsequently translate this knowledge into mechanism-based therapeutics. A promising way to proceed is revealed by the recent studies of rare subsets of ASDs. In this review, we summarize the latest advances in the mechanisms and emerging therapeutics for a rare single-gene ASD, Rett syndrome.

Recent findings
Rett syndrome is caused by mutations in the gene coding for methyl CpG-binding protein 2 (MeCP2). Although MeCP2 has diverse functions, examination of MeCP2 mutant mice suggests the hypothesis that MeCP2 deficiency leads to aberrant maturation and maintenance of synapses and circuits in multiple brain systems. Some of the deficits arise from alterations in specific intracellular pathways such as the PI3K/Akt signaling pathway. These abnormalities can be at least partially rescued in MeCP2 mutant mice by treatment with the therapeutic agents.

Summary
Mechanism-based therapeutics are emerging for single-gene neurodevelopmental disorders such as Rett syndrome. Given the complexity of MeCP2 function, future directions include combination therapeutics that target multiple molecules and pathways. Such approaches will likely be applicable to other ASDs as well.

Keywords
molecular signaling pathways, monogenic disorders, neurodevelopmental disorders, pharmacological therapeutics

INTRODUCTION

Autism spectrum disorders (ASDs) are a group of neurodevelopmental disorders that share common core symptoms of deficits in language and communication, impaired social interactions, and stereotypic or repetitive behaviors. ASDs are characterized by tremendous heterogeneity in their clinical diagnosis and pathophysiological mechanisms. Although all ASD diagnoses share the core symptoms above, individuals with ASD show great differences in severity across these domains and may also suffer from secondary symptoms such as various degrees of cognitive impairment that are specific for each disorder. Similarly, ASD causality is diverse and existing evidence points to a complex genetic basis.

Both ‘rare’ and ‘common’ genetic variants are considered to be risk factors for ASD. Large copy number variants (CNVs) have been recently implicated in subsets of ASDs [1], with specific recurrent CNVs observed across various phenotypes. With few exceptions, CNVs affect several genes and are extremely rare for any given locus. However, many neurodevelopmental disorders with overlapping diagnosis to classic autism, often accompanied by cognitive impairment, are caused by single-gene mutations. Although monogenic disorders account for a small percentage of the total cases diagnosed with autism (Table 1), they might be very informative about the role of common pathogenic pathways affected by the single-gene changes, and hence could be of importance for the discovery of potential...
therapeutic targets (reviewed in [2]). What the vast majority of single-gene ASDs have in common is that the affected gene product is involved in one or more signaling pathways and therefore exerts influence over discrete effectors. In many instances, however, it may be difficult to pin down the defective gene product to a specific symptom and unlikely that a single targeted molecule would restore regular function in the organism. Crucially, single-gene mutations provide accurate mouse models of ASD, which are central for assaying molecular, circuit, and behavioral functions and for developing therapeutics.

Because ASDs are considered to be neurodevelopmental disorders, an important variable to be considered in the development of therapeutics is the time point of intervention. Several kinds of evidence point to the possibility that although early intervention for ASD is likely to have the most impact, therapeutics may still be effective at a range of ages. The development of brain modules, circuits, and synapses, particularly those mediating complex behaviors, has multiple and diverse time courses of maturation and plasticity. Furthermore, the view of ASDs as purely ‘developmental’ disorders is being challenged by the findings that the gene products and molecules affected by the specific disorders are required for appropriate function well past the early development and even throughout life, for deletion of relevant genes in adulthood still leads to the expression of the phenotype [3,4]. The goal of mechanism-based therapeutics is to take advantage of the growing knowledge of development and plasticity of circuits and synapses, and the function and impact of disease genes, in order to intervene at appropriate processes and levels.

We will focus in this review on Rett syndrome, a monogenic X-linked neurodevelopmental syndrome with significant phenotypic overlap with ASD. Rett patients, who are almost always women, develop normally until 6–18 months of age, but suffer from a progressive loss of developmental milestones similar to that observed in regressive forms of autism, such as cognitive and communication deficits, anxiety, irritability, and repetitive behaviors. In addition, the syndrome is characterized by severe motor, sensory, and autonomic nervous system disturbances, such as irregular breathing and heart rate. Other symptoms include seizures, growth failure, and gastrointestinal problems.

**MECP2 AS A HIGH-LEVEL GENOMEWIDE REGULATOR**

About 90% of Rett syndrome cases are caused by mutations in the gene methyl CpG-binding protein 2 (MECP2) [5]. MeCP2 is a highly conserved basic nuclear protein with very diverse functions (Fig. 1a). It was initially found to be associated with methylated DNA, binding to methyl CpG islands especially if AT-rich sequences are adjacent to them [6]. A transcriptional repressor domain associated with the Sin3A–HDAC1–HDAC2 co-repressor complex that blocked mRNA transcription was also identified, so MeCP2 was originally thought to be solely a chromatin-silencing regulator [7]. However, recent evidence has shown that it plays a far more complex role as it might also act as a RNA-splicing modulator and transcriptional activator by binding to CREB [8,9]. This would explain the fact that transcriptional profiling does not show dramatic changes of gene expression in the brain of mutant MeCP2 mice as expected from a putative genomewide regulator.
FIGURE 1. MeCP2-mediated molecular mechanisms that provide opportunities for therapeutics. (a) Schematic of different mechanisms by which MeCP2 can affect gene expression. (b) Schematic showing how MeCP2 regulates protein-coding genes and miRNAs, so as to influence local translation and synaptic development, plasticity, and function. Also shown are attempted targets and molecules for therapeutic interventions. MeCP2 is known to increase the transcription of BDNF which directly increases PI3K/Akt/MAPK signaling, or affect plasticity-related miR-132, which in turn can target MeCP2 expression, thus forming a regulatory loop. In addition, BDNF can activate CREB, which can bind to IGF-1 promoter and regulate transcription. IGF-1 is another positive regulator of the signaling pathways implicated in translation and synaptic plasticity. Akt/MAPK and mTOR are further involved in a double-negative inhibitory loop with miR-199a, a miRNA inhibited by MeCP2. (c) Therapies aimed at restoring proper synaptic function by neurotransmitter rebalancing. BDNF, brain-derived neurotrophic factor; IGF-1, insulin-like growth factor 1; MeCP2, methyl CpG-binding protein 2.

repressor [9,10], and that increased expression levels of MeCP2 are equally detrimental [11]. The role of MeCP2 in epigenetic regulation is expanded further by the finding that MeCP2 can globally alter the state of chromatin condensation [12].

Furthermore, MeCP2 is able to activate retrotranspon element transcription in neurons [13] and promote gene imprinting [14], thus inserting additional layers of complexity to the role of MeCP2 in brain function [15]. In addition, MeCP2 has been recently shown to regulate the expression of microRNAs (miRNAs) important for brain development and plasticity [16–18]. One specific family of MeCP2-regulated miRNAs is miR-132/miR-212 which is known to affect experience-dependent cortical plasticity [19,20]. Intriguingly, miR-132/ miR-212, brain-derived neurotrophic factor (BDNF), and CREB signaling (Fig. 1b) are involved in a positive feed-forward loop [16,21,22]. CREB-binding elements exist in the promoter of IGF-1 which in turn can also activate CREB-mediated BDNF expression [22], thus forming a complex regulatory system representative of the bi-directional interactions between MeCP2 and a subset of its targets. Both BDNF and IGF-1 can directly activate PI3K/Akt/mTOR signaling. In addition, MeCP2 knockout mice also express high levels of the miR-199 family [17,18], that is involved in a mutually inhibitory loop with the PI3K/Akt/mTOR pathway [23,24], which as discussed below is known to be affected in the disease (Fig. 1b). Therefore, MeCP2 is able to modify a subset of PI3K/Akt regulating microRNAs, thus controlling indirectly a large array of molecules responsible for local protein synthesis and synaptic plasticity (Fig. 1). Future work is needed to elucidate whether this proposed mechanism is of importance for Rett syndrome and whether it could provide insights for novel therapeutic options. Moreover, MeCP2 targets can in turn modify other microRNAs, so that we cannot rule out the possibility of MeCP2 further controlling a larger array of molecules. Lastly, MeCP2 mediates the activity-dependent derepression of miR-184 in cortical neurons [25]. Notably, this activity-dependent dissociation of MeCP2 from miR-184 promoter occurs only in the paternal chromosome, thus linking MeCP2 to imprinting of small noncoding RNAs.

MeCP2 is ubiquitously expressed, but higher protein levels are found in the brain [7]. Within the brain, neurons were the first cell population where MeCP2 was detected, but several recent studies have revealed the presence and functional role of MeCP2 in astrocytes [26–28], microglia [29], and neuronal subsets, including inhibitory...
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Interestingly, MeCP2 has been shown to bind to the promoter of somatostatin (SST) and positively regulate its expression together with CREB [18]. It would be therefore worthwhile to study the effects of specific inhibitory cell-type-specific deletions of MeCP2, including SST. In addition to its spatial expression pattern, MeCP2 has a temporal expression regulation in the brain that directly correlates with postnatal neuron and circuit development, peaking postnatally, but remaining high into adulthood. This suggests that MeCP2 is involved in the maturation of existing neurons, rather than in just the differentiation and development of neuronal precursors or neurons [12,31,32], and also that its expression is required throughout life as recent evidence has shown [4].

**FUNCTIONAL MECHANISMS OF MECP2 AND THE CHALLENGES FOR THERAPEUTICS**

As mentioned above, MeCP2 expression peaks during early postnatal development in mice and humans [33], a period when maturation and refinement of synapses and circuits takes place in the cortex. MeCP2 mutant mice have immature excitatory synapses in the cortex and hippocampus [34–36]; furthermore, visual cortical circuits show prolonged plasticity into adulthood, indicating that synapses and circuits fail to mature [34]. The nature and extent of the influence of MeCP2 on synaptic maturity and plasticity are still being investigated. So far, no major circuit connectivity changes have been consistently detected in human Rett syndrome brains or in mouse models [37,38]. This, in conjunction with the reversibility of some of the symptoms in mouse models [34,39,40,41,42,43], might imply that the deficits are at the microcircuit level, involving synaptic transmission and synaptic structural reorganization. In fact, therapies tested to ameliorate Rett syndrome revolve around the restoration of synapse and circuit function with drugs that affect the molecular mechanisms of synaptic maturation [34,41].

BDNF is one of the first recognized direct targets of MeCP2 [44]. MeCP2 control over BDNF expression has proven to be complex, implicating different binding sites in the BDNF gene and partners [45], but there is convincing evidence that the level of BDNF is downregulated in MeCP2 mutant mice in different brain regions [9,44] and its re-expression is beneficial [44]. Not surprisingly, BDNF was one of the first target candidates for a potential therapy. Unfortunately, its therapeutic effects are compromised by its limited capacity to cross the blood–brain barrier, which has prompted the use of BDNF receptor (TrkB) agonists [41,46] or sphingosine-1 phosphate receptor modulator (Fingolimod), which increase BDNF levels in vivo and the activation of its downstream signaling pathway MAPK [47].

Another strategy to address this problem is to use insulin-like growth factor 1 (IGF-1) and its tri-peptide form ([1–3]IGF-1) [34,48]. Both IGF-1 and BDNF supplementation therapies are based on their effects on neuronal developmental, synaptic maturation, and plasticity exerted through two key cell-signaling pathways, PI3K/Akt and MAPK [49,50]. These signaling pathways are known to be disrupted in MeCP2 mutant mice [41,51], and have been directly involved in the regulation of protein translation and neural function by promoting the synthesis of postsynaptic proteins such as PSD-95 [52]. Although there is not a complete picture of how MeCP2 can control the molecular and cellular changes that translate into deficits in synaptic maturation and circuit connectivity, the PI3K/Akt and MAPK pathways should be considered as important players through their control over dendritic and spine genesis, and local protein synthesis [53,54]. In line with the importance of therapies aimed to favor a dynamic and appropriate microcircuit establishment, Rho-GTPases, a family of proteins involved in actin assembly and remodeling, are another potential candidate for therapeutic action in Rett syndrome [55]. This superfamily of proteins is known to specifically control neurite outgrowth and differentiation, spine genesis, and synapse development (reviewed in [56]).

Rett syndrome has a high prevalence of autonomic dysfunction with a remarkable respiratory and cardiac endophenotype. The control of respiration requires a concerted interplay between several neurotransmitters across distinct brainstem nuclei to maintain a normal breathing pattern. Rett syndrome has been characterized by excessive excitatory activity in expiratory neurons and deficits in medulla of nor-epinephrine and serotonin, which contribute to breathing regularity [57,58]. Several therapies have been designed to improve this imbalance (Fig. 1c) variably by increasing GABA availability through GABA reuptake inhibitors, increasing excitatory neuron inhibition by serotonin agonists, or directly increasing norepinephrine and serotonin concentrations with the serotonin norepinephrine reuptake inhibitor Desipramine [59,60]. In addition, a recent study suggests a potential therapy to mitigate arrhythmic cardiac function expressed as prolonged QT intervals, which is another aspect of Rett Syndrome. The sodium-channel-blocking antiseizure drug phenytoin reduces this interval as well as the general occurrence of arrhythmias [61].
Other therapies that have proven to be partially effective in ameliorating some of the deficits in the mutant mouse models and patients include supplementing with l-carnitine, an important antioxidant that improves mitochondrial function [62,63]. Oxidative stress and mitochondrial dysfunction have been recently reported to be increased in the hippocampal cells of MeCP2 mutant mice [64,* reviewed in 65]. However, the most impressive rescue of the phenotype of MeCP2 mutant mice was recently observed following bone marrow transplant from wildtype to mutant mice, which resulted in the engraftment of wildtype microglia into the brain of mutant mice [66**]. Nevertheless, the need for exposure to high doses of radiation so as to disrupt hematopoiesis in the mutant mice and to permeate the blood–brain barrier, perhaps, limits the potential applications of this therapeutic option to treat Rett syndrome patients.

CONCLUSION

MeCP2 has complex functions and mechanisms of action. The transcriptional modulatory polyvalence of MeCP2, its heterogeneity of spatial and temporal expression, and its genome-wide binding interactions make it unlikely that a single-target therapeutic agent will suffice to completely restore physiological functions in Rett syndrome patients. It is, therefore, crucial to identify combinatorial therapeutic approaches and test them in mouse models of the disorder, so that multiple molecular players and pathways affected in Rett syndrome are simultaneously restored, thus increasing the chances of a strong therapeutic response.

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Conflicts of interest

No conflicts of interest.

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- of special interest
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Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

25. Fornai F, Milazzo M, Chiecò P, Gramantieri L. miR-199a-3p regulates mTOR and the actin cytoskeleton, the main targets of the intracellular glucose transporter GLUT4. Proc Natl Acad Sci USA 2008; 105:975–980.
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48. Fingolimod increases the levels of BDNF and MAPK in a mouse model of Rett syndrome. 849.05, Society for Neuroscience Meeting; 2011.


68. Fingolimod increases the levels of BDNF and MAPK in a mouse model of Rett syndrome, improving lifespan and motor skills. This is an approved drug for multiple sclerosis and also crosses the blood–brain barrier.


This study demonstrates the direct relationship between signaling pathways and development of symptoms in a mouse model of Rett syndrome.
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