Mutations in the X-linked gene MECP2 (methyl CpG-binding protein 2) are the primary cause of the neurodevelopmental disorder RTT (Rett syndrome), and are also implicated in other neurological conditions. The expression product of this gene, MeCP2, is a widely expressed nuclear protein, especially abundant in mature neurons of the CNS (central nervous system). The major recognized consequences of MECP2 mutation occur in the CNS, but there is growing awareness of peripheral effects contributing to the full RTT phenotype. MeCP2 is classically considered to act as a DNA methylation-dependent transcriptional repressor, but may have additional roles in regulating gene expression and chromatin structure. Knocking out MeCP2 function in mice recapitulates many of the overt neurological features seen in RTT patients, and the characteristic postnatally delayed onset of symptoms is accompanied by aberrant neuronal morphology and deficits in synaptic physiology. Evidence that reactivation of endogenous MeCP2 in mutant mice, even at adult stages, can reverse aspects of RTT-like pathology and result in apparently functionally mature neurons has provided renewed hope for patients, but has also provoked discussion about traditional boundaries between neurodevelopmental disorders and those involving dysfunction at later stages. In the present paper we review the neurobiology of MeCP2 and consider the various genetic (including gene therapy), pharmacological and environmental interventions that have been, and could be, developed to attempt phenotypic rescue in RTT. Such approaches are already producing valuable insights into the potential tractability of RTT and related conditions, and are useful pointers for the development of future therapeutic strategies.

Key words: gene therapy, methyl CpG-binding protein 2 (MeCP2), neurodevelopment, pharmacotherapy, Rett syndrome.

INTRODUCTION

RTT (Rett syndrome) is a paediatric neurological disorder with a delayed onset of symptoms and is a leading cause of severe mental retardation in girls. First described in the 1960s by Andreas Rett [1] and thereafter by Bengt Hagberg et al. in 1983 [2], RTT was shown in 1999 to be caused primarily by mutations in the X-linked gene MECP2 [encoding MeCP2 (methyl CpG-binding protein 2)] [3]. After a number of groups had developed MeCP2-knockout mouse models and shown their utility in modelling the disorder, a number of attempts to reverse the signs after onset, or to prevent onset, were made. The initial successes reported have demonstrated the tractability of the phenotype and highlighted the potential for treatments for Rett patients that address early stages in the pathogenetic pathway and go beyond merely ameliorating the downstream consequences. It is thus timely to review the nature of the changes seen in the RTT/MeCP2-knockout phenotype and the causal pathways leading to those changes, to lay out the types of therapeutic intervention that could disrupt the pathogenetic pathway, and to review progress in designing such interventions, particularly those aimed at reversing the phenotype.

MeCP2 AND RTT

RTT (MIM 312750) is traditionally thought of as a neurological disorder and is a primary cause of severe mental retardation in girls with an incidence of approximately 1 in 10000 female births [4]. RTT is characterized by its almost exclusive occurrence in females, by onset of overt signs several months postnatally and by a constellation of clinical features [5]. These features, which distinguish RTT from the autism spectrum disorders that they are often co-classified with, include a highly characteristic developmental regression, with accompanying loss of hand skills, impaired mobility and speech, and development of stereotypical hand movements. During regression, social interaction deficits (with features reminiscent of autism) are common. Associated features, such as microcephaly, respiratory/autonomic abnormalities, seizures, scoliosis, growth deficits and early hypotonia, are very prevalent. Some of the associated features may be a direct result of the primary CNS (central nervous system) deficits, but several may also be influenced by peripheral effects. Clinical presentation and severity show quite a wide range of variation, and patients may exhibit all of the essential features necessary for the RTT diagnosis (typical RTT), or they may show differences that enable their assignment to one of a range of atypical RTT diagnoses [5].

RTT cases are usually the result of dominantly acting de novo [6] mutations in the X-linked gene MECP2, which encodes MeCP2. More than 600 pathogenic MECP2 mutations have been reported (RettBase; http://mecp2.chw.edu.au/mecp2/), including missense, nonsense, frameshift and large deletion mutations. Most pathogenic mutations in MECP2 cause RTT in heterozygous females [7], but a range of MECP2 mutations associated with other phenotypic outcomes, including milder forms of learning disability and, rarely, autism, are also known [8]. Boys inheriting

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**Conflict of interest**

There is no conflict of interest.

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**Abbreviations**

AAV, adeno-associated virus; ACh, acetylcholine; AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid; BBB, blood–brain barrier; BDNF, brain-derived neurotrophic factor; CaMKII, Ca2+/calmodulin-dependent protein kinase II; CNS, central nervous system; EE, environmental enrichment; GABA, γ-aminobutyric acid; IGF1, insulin-like growth factor 1; MeCP2, methyl CpG-binding protein 2; NMDA, N-methyl-D-aspartate; PSC, premature stop codon; RTT, Ret syndrome; siRNA, small interfering RNA; XCI, X chromosome inactivation.

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a mutant MECP2 allele that would normally cause typical RTT in a female are much more severely affected, presenting with infantile encephalopathy and usually not surviving infancy. The differences between the observed phenotypes in males and in females are explained in terms of the proportion of cells in the nervous system expressing the mutant allele. Although all MeCP2-containing cells will express the mutant allele in males that have inherited a single mutant X chromosome, the female brain, due to random X-chromosome inactivation, will develop into a mosaic network of cells with some expressing the mutant allele and others expressing the normal allele. In this way the pathology associated with the mutant allele is diluted (at the network level) in the female brain, albeit for direct cell-autonomous actions of the mutation.

**FUNCTION OF MeCP2**

MeCP2 is predominantly a nuclear protein that was first discovered through its affinity for DNA sequences containing methylated 5′-CpG-3′ dinucleotides [9]. It is a member of a small family of MBD (methylated DNA-binding domain) proteins, some of whose members can act as transcriptional repressors [10]. MeCP2 is expressed quite widely throughout the body, with notably high expression in postnatal neurons [11–13]. It is expressed as two major splice variant isoforms, e1 and e2, that encode proteins with different N-termini (see Figure 1). Both isoforms are thought to have two primary functional domains, a methyl CpG-binding domain and a transcriptional repressor domain that interacts with, among other things, the Sin3a repressor complex [14]. Previous biophysical studies have probed the binding specificity of MeCP2 and have confirmed the interaction (via hydration within the major groove) with methylated DNA and also the interaction with nucleosomes [15,16]. Despite this knowledge, the precise biological function of MeCP2 remains unclear. Proposed additional or alternative functions include selective enhancement/activation of gene expression [17], chromatin regulation [18] and RNA processing [19,20], among others. It has previously been established that MeCP2 is distributed across the genome very much in parallel with methylation density and to the exclusion, in neurons, of histone H1 [21]. These data suggest that MeCP2 plays a major role in the suppression of transcription throughout a very wide genomic distribution; in this way it may be best to describe the function of MeCP2 in terms of global dampening of transcriptional noise.

**RTT-like features in Mecp2 mutant mice**

The status of RTT as an intriguing, relatively common, monogenic disorder has engendered considerable interest in investigating the underlying pathology. A large part of this interest stems from a desire to develop rational therapies for patients, but it has also emerged that gaining a fuller understanding of the underlying pathology and neuronal dysfunction in RTT may provide insights into the pathophysiology of neurodevelopmental disorders more generally [22]. Since most MECP2 mutations leading to RTT involve loss-of-function of the mutant allele, RTT can be modelled using Mecp2-knockout mice.

Several of the models that have been created recapitulate many of the cardinal features that characterize RTT in humans, although there are differences [23–27], and reflect something of the phenotypic variability seen in patients. In most of the models, the hemizygous null males demonstrate onset of overt signs at approximately 5–6 weeks of age (equivalent to early
MeCP2 and Rett syndrome

By activating/restoring gene function in different cell types/regions and at different times we can establish the requirement for MeCP2 expression and assess the potential for preventing/reversing the phenotype associated with lack of MeCP2. Early neuron-specific and early global expression of MeCP2 have both prevented the onset of phenotype, whereas delayed global reactivation in symptomatic mice has resulted in a significant improvement in RTT-like phenotypes. Chao, 2010 [33]; Samaco, 2009 [34], Luikenhuis, 2004 [26], Giacometti, 2007 [63], Guy, 2007 [23].

adulthood, developmentally), in marked contrast with humans, where hemizygous males have onset of overt signs at or shortly after birth. The similarities emerge when considering progression, however, which in the mice is usually fairly aggressive, with death at 10–20 weeks. After onset, the males develop motor impairment, tremor, breathing abnormalities and limb stereotypes [23–25]. In heterozygous female mice, overt onset is considerably delayed, occurring at approximately 6 months of age (considerably later than in human females, in developmental terms), and there is much slower progression, with stabilization of the phenotype and an apparently normal lifespan. Thus the phenotype associated with most of the mouse models can be viewed as being a little milder than that of RTT and other consequences of MECP2 mutation.

There is some degree of genotype–phenotype correlation; models with MeCP2 protein-null phenotypes [24,25], for example, are more severe than models in which late-truncated forms of MeCP2 are expressed [28].

MeCP2 is expressed in a range of tissues [11,13,27], but is especially abundant in post-mitotic neurons. Why a mutation in a widely expressed protein produces a syndrome with a predominantly neurological phenotype is a key question that remains unanswered. One possibility is simply that lack of MeCP2 may be more detrimental in cell types in which it is normally abundant, presumably because, in this case, neurons are highly sensitive to the removal of a regulator of an as yet unidentified process enriched in those cells. Recognition that processes in glia may also contribute to the phenotype, despite the lower expression of MeCP2 in these cells, is becoming more widespread [29,30]. The role of post-translationally modified forms of MeCP2 has yet to be confirmed, but the observation that phosphorylation of MeCP2 at Ser421 occurs exclusively in the brain and not in peripheral tissues [11] is interesting. Whatever the case, it is evident that mice lacking MeCP2 in neurons only do show overt RTT-like symptoms, whereas Mecp2-knockout mice in which the expression of exogenous MeCP2 is driven in neurons alone show an apparently normal phenotype [26], suggesting that the bulk of the observable knockout phenotype comes from effects in neurons. In Figure 2, we review past and ongoing interventions designed to probe the developmental stage at which MeCP2 is most crucial, and the critical cell- and tissue-types likewise. The results of these studies so far point to the strong possibility that specific aspects of RTT pathology are explained by the lack of MeCP2 in specific neuronal populations.

Figure 2  Mapping the effect of Mecp2 deletion or activation in the mouse

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knockin model, which expresses a truncated form of MeCP2 and displays significantly milder symptoms than in the knockout, male mice show impairments in hippocampus-dependent spatial memory as well as social memory [28]. A recent study in which Mecp2 was silenced specifically in inhibitory GABAergic cells (GABA is γ-aminobutyric acid) revealed a range of subtle RTT-like neuropsychiatric phenotypes, including autistic-like repetitive behaviours [33]. In another study, in which MeCP2 was silenced in tyrosine hydroxylase-containing neurons, mice were found to display motor abnormalities and breathing problems, including an increased incidence of apnoeas, suggesting that dysfunction in amnergic systems may be responsible for some RTT-like breathing phenotypes [34].

CELLULAR ALTERATIONS AND RTT AS A SYNAPTOPATHY

In addition to the neurological and behavioural features that characterize the syndrome, a range of structural changes in the brain and changes in cellular and synaptic physiology are associated both with RTT and with the Mecp2-Knockout phenotype in mice. The most conspicuous features in RTT patients are a reduced brain size and weight, as well as more subtle changes in neuronal packing density and aspects of cellular morphology, such as reduced dendritic branching complexity and changes in spine density and morphology [35,36]. Similar changes are reported in Mecp2-null mice [25] where there is evidence for both cell autonomous and non-cell autonomous alterations in neuronal morphology with respect to the cellular expression of MeCP2 [37]. As well as cellular morphology, the MeCP2 level is reported to regulate the number of excitatory synaptic connections formed [38] and may also affect axonal guidance [39]. Somewhat surprisingly, electrophysiological studies have revealed relatively modest changes in the salient electrical properties of cortical neurons [40], although more pronounced changes are reported in subcortical regions [36]. In contrast, a robust body of evidence exists for changes in synaptic signalling, affecting both excitatory and inhibitory amino acid neurotransmission [40–43]. With respect to inhibitory amino acid neurotransmission, loss of MeCP2 has been shown to reduce quantal GABA release consistent with a presynaptic reduction in neurotransmitter content [33]. This may account for the propensity for seizures to occur in the RTT brain. In addition to changes in the baseline properties (frequency and amplitude) of synaptic events, several studies have reported deficits in both short- and long-term forms of synaptic plasticity [23,28,44,45]. Interestingly, synaptic plasticity appears normal in young Mecp2-mutant mice [23], but shows progressive impairment when tested in older mice upon symptom onset [23,45]. It is clear that RTT can, in part, be considered a synaptopathy (disease of the synapse). However, the exact mechanism by which absence of functional MeCP2 in the nucleus produces a synaptic deficit phenotype remains to be established.

TREATMENT STRATEGIES AND REVERSIBILITY OF THE RTT PHENOTYPE

The orthodox view is that abnormalities in brain development during critical periods of growth and maturation will produce aberrations in the nervous system with resultant complex neurological and psychiatric features, and that these defects are essentially irreversible in adults owing to the limited ability of the brain to generate new neurons or to radically rewire itself. However, a number of studies of animal models of diseases ranging across Down’s syndrome [46,47], neurofibromatosis type 1 [48,49], tuberous sclerosis [50–52], Rubinstein–Taybi syndrome [53,54], fragile X syndrome [55,56] and Angelman syndrome [57] are beginning to demonstrate an unexpected propensity for phenotypic reversal, even in adult mice (reviewed in [58]). This propensity has also previously been reported in RTT [23]. These studies raise the question of what constitutes a neurodevelopmental disorder as well as whether these conditions have both neurodevelopmental components and aspects of dysfunction that are non-development related.

RTT is considered to result either from a failure of neurons to mature (a developmental process), or of their failure to maintain a mature phenotype (a maintenance process). Studies in the olfactory system suggest that MeCP2 may be important in both developmental processes (maturation during synaptogenesis) and maintenance processes in relation to neuronal phenotype [39,59]. Other studies have stressed the maturation role [60]. MeCP2 is also present in astrocytes and other non-neuronal cell types in the brain, albeit at much lower levels [61]. Although a loss of MeCP2 function in astrocytes may lead to altered release of neurotrophic factors and changes to dendritic outgrowth [29,30], and contribute to non-cell autonomous aspects of RTT pathology, deletion and neuron-specific expression of Mecp2 studies in mice show that the dominant mutant phenotype is principally due to the absence of MeCP2 from neurons [24–26].

Despite all of the detectable changes in neuronal morphology and physiology [62], RTT is not considered a neurodegenerative disorder. In considering whether the phenotype is reversible or even preventable, three scenarios must be considered. First, since neurons lacking MeCP2 display long-term survival and since neurons seem to require MeCP2 throughout their lives, it is possible that the introduction of normal MeCP2 or therapeutic strategies targeting MeCP2-related signalling might restore function and thereby reverse deficits seen in RTT. Alternatively, it may be that MeCP2 is essential for neuronal development during a specific time window, after which damage caused by its absence is irreversible. A third scenario combines both of these ideas, whereby certain RTT-like features can be rectified in the mature nervous system if cells begin expressing MeCP2, whereas other features are critically dependent upon the presence of MeCP2 during essential, developmentally inflexible, processes and are thus insensitive to simple restoration of MeCP2 or other intervention beyond a critical period.

WHAT DO MOUSE STUDIES TELL US ABOUT THE TRACTABILITY OF RTT?

The mouse models of RTT have played a conspicuous role in studying the requirement for MeCP2 in different cell types and at different stages of development, as well as in testing the ‘reversibility’ of the RTT-like phenotype (Figure 2). For instance, Luikenhuis et al. [26] showed that severe overexpression of a Mecp2 transgene under a generic neuron-specific (Tau) promoter produced profound motor dysfunction in wild-type and Mecp2-null mice. In contrast, modest overexpression of this transgene in the Mecp2-null mice could prevent the occurrence of the RTT-like phenotype [26]. This experiment was interpreted as providing evidence of a critical requirement for MeCP2 in neurons, but it also emphasized the importance of maintaining the MeCP2 protein expression at an appropriate level, which has therapeutic significance when considering potential gene therapy strategies (see below). In another study, Giacometti et al. [63] tested whether MeCP2 expression in pre- or post-mitotic neurons could have an impact on phenotype progression in Mecp2-null mice. A number of Cre lines were generated so that MeCP2 could be expressed in specific brain regions and at different developmental time points.
(Nestin and Tau promoters were used to mediate activation in the whole brain in pre- and post-mitotic neurons respectively, whereas CaMKII (Ca²⁺/calmodulin-dependent protein kinase II) Cre lines 93 and 159 mediated reactivation of Mecp2 expression in the forebrain, brain stem and hippocampus at postnatal days 21 and 30 respectively). Early brain-specific activation of Mecp2 prolonged lifespan, delayed development of motor deficits and improved motor activities that had been reported in Mecp2-null mice [25]. Reactivation of Mecp2 also restored normal body weight, brain weight and neuronal size, which are typically reduced in the Mecp2-null mouse [25]. On the other hand, delayed forebrain activation of Mecp2 improved locomotor activity, but showed less prolongation of the lifespan compared with global brain activation. These results suggest that the introduction of MeCP2 to the nervous system under artificial promoters is sufficient to enable a modest amelioration of the RTT-like phenotype and prolong lifespan.

In another study, Guy et al. [23] created a mouse model in which the endogenous Mecp2 gene is silenced by insertion of a lox-STOP cassette, but which can be conditionally activated (Cre-mediated STOP cassette excision) following tamoxifen injection. Importantly, this enabled the reactivation of Mecp2 at its endogenous locus and under the control of its own promoter and regulatory elements. This approach resulted in a very robust reversal of the RTT-like phenotype. Hemizygous males, which typically develop early RTT-like symptoms, rapid progression and death after a few weeks, developed no RTT-like signs if tamoxifen treatment was applied in the presymptomatic phase and showed robust symptom reversal, and dramatically enhanced survival if treated once symptoms had developed. Furthermore, heterozygous female mice (the accurate model of RTT in humans) displayed a dramatic improvement in RTT-like phenotype, a normalization of body weight and a restoration of synaptic plasticity deficits, even when treated as fully adult mice (>6 months). These findings confirm the propensity for phenotypic reversal, at least in the mouse. This reversibility may bring into question the categorization of RTT as a neurodevelopmental disorder as it suggests that MeCP2-deficiency during brain development does little lasting damage. However, it remains to be established whether improvement in overt signs (tremor, locomotion, abnormal breathing etc.) is accompanied by improved cognition and other behavioural correlates. Nevertheless, these mouse genetic studies suggest that there is the opportunity, even in the fully mature nervous system, to modulate MeCP2 levels or target downstream processes by pharmacological means to prevent, or reduce the severity of, the condition.

Indeed, recent complementary experiments in an adult onset model of RTT (tamoxifen-induced excision of a floxed Mecp2 allele) suggest that MeCP2 is critical for ongoing neurological function in the adult nervous system and that potential therapies for RTT are likely to be required throughout life [64]. In contrast with the significant reversal seen following a global reintroduction of MeCP2, studies focusing on more restricted expression of Mecp2 and using promoters other than the endogenous Mecp2 promoter have shown a more modest effect. For instance, using two neuronal-specific promoters (CaMKII, forebrain-specific; enolase, striatum/cerebellum-specific) to restore expression of MeCP2 to these regions was unable to prevent the appearance of most RTT-like phenotypes [65]. Jugloff et al. [66], again using the CaMKII promoter, observed improvements in activity, mobility and locomotor activity to wild-type levels in heterozygous Mecp2+/- female mice, but continued impairment in other tests such as central field exploration. It is unclear from these studies whether the sustained deficits result from an inherent irreversibility due to neurodevelopmental aberrations or to dysfunction of regions or cell types in the brain still devoid of MeCP2, or to the exogenous promoters driving cellular MeCP2 expression at levels outside the physiological range, or to other unknown mouse-specific processes.

In addition to targeting Mecp2 itself, genetic approaches in mice have also been used to explore interactions with other signalling processes in the brain. BDNF (brain-derived neurotrophic factor) is a neurotrophic factor whose expression is regulated by MeCP2 protein [67,68]. Neural activity leads to phosphorylation of MeCP2, which correlates with the transcriptional induction of Bdnf. It has been reported that Mecp2-null mice display lower levels of BDNF protein with the onset of RTT-like symptoms and that Bdnf-knockout mice also exhibit some of the RTT-like phenotypes including limb clasping and reduced brain weight [69]. To explore the potential involvement of BDNF in RTT pathogenesis, a Mecp2 and Bdnf double-knockout mouse line was created [69]. This was found to result in an earlier onset of RTT-like features and lethality. In contrast, Mecp2-null mice in which BDNF is overexpressed resulted in a delayed onset of RTT-like signs and a significantly increased lifespan.

**RTT THERAPEUTIC STRATEGIES AT THE LEVEL OF THE GENE**

The demonstrated reversibility of the Mecp2-knockout phenotype in Mecp2-STOP mice [23], as described above, has stimulated a good deal of activity in exploring therapeutic approaches designed both to reverse existing dysfunction in RTT and to prevent its onset. In addition to the question of which of these two basic approaches to take therapeutically, there is also the question of where in the pathogenetic pathway to target the therapy. Two main categories of intervention suggest themselves: targeting the primary underlying cause (i.e. a loss-of-function mutation in MECP2) or targeting processes further downstream in the pathway. In the following sections we review the potential for such therapeutic approaches and describe any progress thus far.

**RTT THERAPEUTIC STRATEGIES AT THE LEVEL OF THE GENE: REACTIVATION OF THE NORMAL ALLELE**

As mentioned above, MECP2 is located on the X chromosome and is subject to XCI (X chromosome inactivation), such that in each cell in a heterozygous female RTT patient expresses only the normal (thus having an MeCP2-positive phenotype) or only the mutant MECP2 allele (thus having an MeCP2-negative phenotype, in the case of substantial loss-of-function mutations), never both. This process is usually random and results in an approximately 50:50 mixture of cells of each type, although ratios may vary somewhat from tissue to tissue and between individuals. Studies of the distribution of brain cells expressing the normal and mutant MECP2 alleles have revealed that in this critical tissue MeCP2-negative cells tend not to be clustered, but intermingling with the MeCP2-positive cells in a fine-scale mosaic pattern [23].

A number of studies have reported skewing of the XCI ratio away from 50:50 in RTT patients [70,71]. It is well known that the rare familial cases of RTT are usually explained by the fact that mothers of these patients carry the causative mutation, but never both. This process is usually random and results in an approximately 50:50 mixture of cells of each type, although ratios may vary somewhat from tissue to tissue and between individuals. Studies of the distribution of brain cells expressing the normal and mutant MECP2 alleles have revealed that in this critical tissue MeCP2-negative cells tend not to be clustered, but intermingling with the MeCP2-positive cells in a fine-scale mosaic pattern [23].

In contrast with the significant reversal seen following a global reintroduction of MeCP2, studies focusing on more restricted expression of Mecp2 and using promoters other than the endogenous Mecp2 promoter have shown a more modest effect. For instance, using two neuronal-specific promoters (CaMKII, forebrain-specific; enolase, striatum/cerebellum-specific) to restore expression of MeCP2 to these regions was unable to prevent the appearance of most RTT-like phenotypes [65]. Jugloff et al. [66], again using the CaMKII promoter, observed improvements in activity, mobility and locomotor activity to wild-type levels in heterozygous Mecp2+/- female mice, but continued impairment in other tests such as central field exploration. It is unclear from these studies whether the sustained deficits
view of the fact that most skewing studies have been conducted in peripheral tissues such as blood with much higher cell turnover and that the ratio may not be the same in affected brain regions (but see [74]). However, there is at least some evidence to suggest that similarly skewed patterns are found in brain in the mouse models [75,76].

One strategy for delivering MeCP2 to those cells expressing the mutant allele could be to encourage the reactivation of the inactive X chromosome to allow expression of the normal allele in the same cells. Several groups are currently tackling this highly challenging approach. Results of studies conducted before the X chromosome inactivation process was understood, suggested that simple chemical interventions (application of 5-azacytidine) designed to reduce genomic methylation levels could result in activation of X-linked genes in cell culture models [77]. However, this approach is unlikely to be a panacea for patients. Among the likely problems that may be encountered are that if the entire inactive X chromosome is reactivated, the X-linked gene dosage problem for which XCI evolved will supervene and pathological levels of gene expression are likely to result at many loci. If the reactivation can be targeted only to the MECP2 locus or the immediate vicinity, that might avoid the general dosage problem; however, there are currently no obvious resources that can be used to target reactivation in this way, and neither are there any ways to target only those cells that have inactivated the normal allele.

**Figure 3  Challenges associated with gene therapy: delivery, dosage and mosaicism**

(A) Levels of endogenous MeCP2 vary between cell types, but knockout and overexpression studies suggest that levels are important and that both too little or too much MeCP2 may be detrimental to cell function. (B) Females with RTT are heterozygous for the X-linked mutation giving rise to a mosaic pattern of cells expressing the functional allele and cells expressing the mutant allele. Poorly regulated gene delivery may result in a proportion of transduced cells having very different levels of functional MeCP2. (C and D) Strategies to prevent overexpression of MeCP2 in RTT females, by designing therapies to deliver functional MECP2 while ensuring that exogenously derived MeCP2 is not added to the pool of endogenous cellular MeCP2. (C) Endogenous MeCP2 could be used to silence transgene expression through incorporation of an efficient target for its binding, leading to repression of the transgene. (D) A suppression and replacement strategy tailored for X-linked genes. Both normal and mutant endogenous alleles are silenced using siRNA, whereas the transgene supplying MeCP2 is tailored to be insensitive to the siRNA construct (*) in D. Thus all transduced cells now express only the transgene.

RTT THERAPEUTIC STRATEGIES AT THE LEVEL OF THE GENE: GENE THERAPY

Another approach at the level of the gene is gene therapy, for which several avenues are open. A major objective might be to deliver a working copy of MECP2 to as many affected cells (i.e. brain neurons) as possible and raise function (at both the molecular and cellular level) above a threshold required for improvement of the clinical picture. Challenges to the probable success of such approaches include finding an appropriate vector, transducing sufficient cells, avoiding transgene repression and avoiding overexpression of exogenous MeCP2 (Figure 3).

Given that the RTT phenotype results from effects on postmitotic neurons with long lifespans and cannot simply be fixed by delivery of cells with the right characteristics into the tissue, it is clear that if gene therapy is to work it will have to involve in vivo delivery, which is not a trivial undertaking. Vectors currently in use are largely based on lentiviruses, retroviruses and AAV (adeno-associated virus). Several groups are actively developing such tools for delivery of genes encoding MeCP2, particularly for use in the mouse models described above, but to date there are no reports that viral vector-based delivery of Mecp2 to the brain of Mecp2-null mice can rescue any aspects of the RTT phenotype. A preliminary attempt to use adenovirus to deliver a construct driving MeCP2 expression in the striatum of Mecp2-null mice reported decreased motor deterioration and restoration of voluntary motor activity [78]. However, this has never been followed up, reported in detail or repeated. Rastegar et al. [79] demonstrated the potential for lentiviral transgene delivery to improve the phenotype of Mecp2-null neurons derived from neuronal stem cells in culture. These neurons displayed more dendritic growth and branching than uninfected controls. The same authors also showed the endogenous Mecp2 promoter to be a sensible choice in driving cell type-appropriate expression and thus avoiding overtly ectopic patterns of expression [79].

The requirement mentioned above of keeping MeCP2 expression levels within a window for normal function is one of the more obdurate challenges (Figure 3A). There is evidence that elevated levels of MeCP2 may be detrimental, since patients with
duplication of Xq28 in regions spanning the MECP2 locus show a range of neurological features including altered head growth rates, ataxia, seizures and mental retardation [80–82]. Similarly, mice overexpressing MeCP2 show behavioural changes. Although mice with modest overexpression of MeCP2 show enhanced motor co-ordination, a reduced anxiety phenotype and increased context-dependent fear conditioning [83], mice expressing higher (2–4-fold) levels of MeCP2 in neurons display tremors and motor dysfunction [26]. In the mouse Mecp2 reactivation study [23] it was assumed that removal of the STOP cassette would not have a large effect on later expression levels of MeCP2 driven by its endogenous promoter and regulatory elements, and that expression would effectively be at wild-type levels in each rescued cell. Assuming that newly synthesized MeCP2 molecules (in a previously MeCP2-negative cell) are able to adopt the normal distribution within the nuclear chromatin, establish their normal suite of roles (including interpreters of the DNA methylation signal) and correct dysfunction at a single-cell level, the level of overall phenotypic rescue at the organismal level would then be determined largely by the proportion of cells of critical cell types rescued in the brain. In the reactivation study, efficiency of reactivation in the brain was approximately 80%, suggesting, in mice at least, that this level of cellular rescue will produce a substantial improvement in RTT-like phenotypes. The Mecp2-STOP model was engineered to enable the controlled activation of Mecp2. However, a similar strategy cannot be applied in RTT patients as there is no way to alter, reliably, the MECP2 gene sequence in large numbers of cells in situ in the brain. Gene therapy approaches will therefore have to deliver an extra copy of MECP2 to each cell that becomes transduced, while avoiding a significant multiplicity of infection, which could lead to large numbers of cells overexpressing MeCP2 at high levels (see Figure 3B).

Even in cells transduced by a single virus particle and expressing MeCP2 from a single extra copy of the gene, the fact that female patients (and heterozygous mice) are a mosaic of MeCP2 expressing and non-expressing cells due to XCI itself causes problems. Virus particles delivering MeCP2-expressing constructs to cells expressing the mutant allele are unlikely to cause problems, but those delivering constructs to cells expressing the wild-type allele could cause overexpression (see Figures 3C and 3D). Two strategies to avoid this scenario have been devised. In one strategy (illustrated generically in Figure 3C), the construct itself is designed in such a way that pre-existing expression of MeCP2 leads to the transgene not being expressed. One potential means for doing this is to design a highly efficient MeCP2-binding target into the promoter driving transgene expression in the hope that this would stimulate heterochromatin formation and long-term repression of the transgene. In cells expressing the mutant allele, this shutdown would not occur until sufficient transgenic MeCP2 had built up in the cell to produce a negative-feedback effect on its own expression.

The other strategy, employed by Zhou et al. [11], is to design a construct that includes an agent that will suppress endogenous MeCP2 expression while leaving the transgene to do its work (see Figure 3D). A polycistronic construct incorporating a siRNA (small interfering RNA) that will suppress both wild-type and mutant MeCP2 translation from endogenous mRNA was designed. Base changes in the sequence of the Mecp2 transgene in the same construct ensured that translation of the mRNA from exogenous Mecp2 transgene was not suppressed. Both of these approaches can be seen as varieties of the ‘suppression and replacement’ strategy currently being developed for a range of dominant disorders, but tailored for use in relation to an X chromosome-linked gene.

An additional challenge is finding the most appropriate vectors for delivery of gene therapy constructs, as each of the vectors currently in use has limitations. Lentiviral vectors are ideal from the point of view of their ability to transduce a wide range of non-dividing cells, their high neuronal infection efficiency, and their ability to drive stable long-term expression from constructs integrated into the host cell genome [84]. Their other characteristics are less ideal; they cannot pass the BBB (blood–brain barrier) even after mannitol disruption [85] so have to be delivered by direct injection into the brain parenchyma where they have limited capacity to spread beyond the injection site [86], and there are inevitable safety issues surrounding their potential to cause insertional mutagenesis and tumorigenesis.

AAV vectors have become an attractive alternative to lentiviral vectors [87]. AAV-based strategies for gene therapy of cystic fibrosis [88], haemophilia B [89], α1-antitrypsin deficiency [90], neurodegenerative lipid storage disorders [91] and Parkinson’s disease [92] are at the advanced pre-clinical or Phase I trial stage. Different serotypes (with coat protein from different viral strains) have been studied for their infection properties and several would be suitable for use in the brain. The AAV9 serotype, particularly, is showing promise as it has displayed high CNS transduction efficiency after systemic administration [93] and is reported to rescue the phenotype in a mouse model of ALS (amyotrophic lateral sclerosis) after intravenous administration [94]. AAV9 is very promising in the case of RTT gene therapy as it can cross the BBB, infect neurons efficiently, mediate long-term transgene expression [95–97] and does not integrate in a random fashion into host chromosomes (the vector DNA usually persisting as an episome in the nucleus) with accompanying safety benefits [98,99]. Another challenge is to get the therapeutic ‘dosage’ right. High viral titres are essential to get good coverage (a high proportion of cells infected), but having more than one viral particle infect each cell could be detrimental, as discussed above. These and many other challenges remain to be surmounted before gene therapy for RTT becomes a reality, but its potential is clear and work in this area is likely to blossom over the next few years.

**PHARMACOLOGICAL APPROACHES IN RTT: CURRENT CHALLENGES**

As an alternative to targeting MeCP2 through gene therapy, many groups have argued that identifying factors that are downstream of MeCP2 function and targeting those pharmacologically is the most sensible approach to developing rational therapies in RTT (Figure 4). It is certainly the case that low-molecular-mass entities (classical ‘drug’ molecules) are unlikely to replace the function of MeCP2. However, the problem at present is that the precise role of MeCP2 is unknown – the widely believed proposition that MeCP2 has distinct ‘target genes’ whose expression becomes significantly aberrant in the absence of functional MeCP2 has little corroborated support [21]. If MeCP2 truly functions to sense/interpret the DNA methylation signal then the action of MeCP2, or more specifically the precise dysfunction caused by its absence, will be uniquely dependent on the methylation status within a given neuronal type or indeed individual neuron. Moreover, the high abundance of MeCP2 together with the ubiquity of DNA methylation suggests that candidates for targeting its core chromatin-binding function therapeutically will be multiple and diverse [21]. That said, several attempts (projects listed at http://www.rsr.org/researchers) are being made to test the effectiveness of existing drug molecules based on our current, rather limited, understanding of the underlying biology of MeCP2 function. Although the availability of accurate animal
Neurotrophic factors

The brain signalling pathway that has received most attention in relation to MeCP2 to date is the neurotrophic factor BDNF. Changes in the levels of BDNF are consistently reported in the RTT brain [67,68] and genetic potentiation of BDNF signalling is reported to ameliorate key symptoms in the MeCP2-null mouse [69]. On the basis of this link between a loss of MeCP2 and synaptic dysfunction and which may represent secondary effects with less value as therapeutic targets. Teasing apart the primary molecular dysfunction in RTT and the important and tractable downstream changes in signalling processes remains an important challenge.

Neurotransmitter systems: monoamines and acetylcholine

Another transmitter system that has attracted attention in RTT is the monoamines. Bioamine levels, including that of the monoamines. Bioamine levels, including that of noradrenaline, serotonin, dopamine and several major bioamine metabolites, are reported to be reduced in post-mortem RTT patients that were then treated with full-length IGF1 [109]. Recombinant IGF1 is available as a clinical formulation and is currently undergoing initial phase clinical trials in RTT patients (http://clinicaltrials.gov/ct2/show/record/NCT01253317?term=rett+syndrome).

Figure 4 Potential genetic and pharmacological intervention strategies for reversing RTT pathologies

Potential therapeutic strategies aimed at targeting the MECP2 gene, mRNA, protein or downstream signalling processes. These strategies are designed to: (1) overcome the effects of nonsense mutations (gentamycin-mediated ribosomal readthrough of PSCs), (2) fold/retold misfolded proteins with missense mutations (chaperone therapy), (3) provide neurons with normal functional MeCP2 protein molecules through activation of the inactive X-chromosome (not feasible at present), and (4) reintroduce a normal copy of MECP2 (viral-mediated gene therapy). Animated versions of steps 1-4 are available at http://www.BiochemJ.org/bj/439/0001/bj4390001add.htm. Expression of functional MeCP2 is expected to result in reversal of neurological features. Other strategies use pharmacological approaches based on (5) existing therapeutic drugs that target neurotransmitter systems with aberrant functioning in RTT patients and MeCP2 mutant mice, or on (6) neurotrophic factors that boost functioning of neuronal network systems. (7) Existing symptomatic therapies target specific symptoms such as seizures. AEDs, antiepileptic drugs.

models of RTT is a great asset for testing the effectiveness of pharmacological strategies, the main difficulty is that there exists no effective system for target identification. Furthermore, it is unclear which of the various reported changes in gene expression, brain neurochemistry, neuronal/synaptic morphology, cellular/synaptic function and network electrical activity patterns [100–102] in the RTT brain are a primary result of MeCP2 dysfunction and which may represent secondary effects with less value as therapeutic targets. Teasing apart the primary molecular dysfunction in RTT and the important and tractable downstream changes in signalling processes remains an important challenge.

Neurotrophic factors

The brain signalling pathway that has received most attention in relation to MeCP2 to date is the neurotrophic factor BDNF. Changes in the levels of BDNF are consistently reported in the RTT brain [67,68] and genetic potentiation of BDNF signalling is reported to ameliorate key symptoms in the MeCP2-null mouse [69]. On the basis of this link between a loss of MeCP2 and BDNF dysregulation, Ogier et al. [103] tested the ampakine CX546, a positive modulator of AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors and a known enhancer of BDNF levels, in Mecp2-null mice. This study focused on respiratory function and described the restoration of normal breathing patterns and respiratory minute volume upon CX546 treatment. This is a significant finding as respiratory abnormalities are an especially problematic feature of the clinical picture in RTT [104]. Although different ampakines vary in their effectiveness to potentiate BDNF, the study is nevertheless consistent with the hypothesis that elevations of BDNF are associated with improved symptoms and supports the concept of targeting BDNF signalling in RTT. In a follow-up study, Kline et al. [41] showed that BDNF levels were especially reduced in a brain stem nucleus important in cardiorespiratory control and that the reduced BDNF availability resulted in aberrant synaptic signalling. The synaptopathies in the MeCP2 mutant brain were reversed by subsequent application of exogenous BDNF, perhaps providing a cellular correlate by which boosting BDNF may re-establish normal homoeostatic control. It will be interesting to find out whether ampakines or novel TrkB (tropomysin receptor kinase B) receptor ligands may improve other features of the MeCP2-null phenotype. Analogous studies in a knockin model of Huntington’s disease show that ampakine-induced BDNF up-regulation could reverse deficits in synaptic plasticity and improve impairments in long-term memory, but had no detectable effect on locomotor activity impairments [105].

Another growth factor in use is a derivative of IGF1 (insulin-like growth factor 1). Full-length IGF1, which is important in neuronal maturation and as a regulator of synaptic plasticity, is widely expressed in the brain and signals through a receptor tyrosine kinase to regulate neuronal survival and synaptic maturation [106]. Mecp2-null mice and RTT patients have elevated levels of the IGF-binding protein 3, the consequence of which is predicted to be an overall reduction in IGF1 signalling [107]. Tropea et al. [108] have capitalized on observations that the N-terminal tripeptide of IGF1 (known as GPE; its effects are mediated via a route that does not involve the IGF1 receptor complex) has neuromodulatory and neuroprotective effects, and have tested whether systemic administration of the GPE tripeptide can affect dendritic and synaptic maturation and reverse key aspects of the RTT phenotype in Mecp2-null mice. At the cellular level, the GPE tripeptide was found to increase PSD-95 (postsynaptic density 95; a major postsynaptic density protein and synaptic marker) labelling, cortical spine density and produce a very modest, but significant, increase in the amplitude of excitatory synaptic currents. GPE tripeptide treatment partially restored brain weight towards wild-type levels and at the organismal level the authors report a significant increased lifespan, improved locomotor activity and more modest improvements in cardiac and respiratory function. However, treated mice went on to ultimately develop all of the characteristic RTT-like features and showed a much reduced survival compared with control mice. In another study, an increase in the number of glutamatergic synapses was observed in neurons differentiated from induced pluripotent stem cells taken from RTT patients that were then treated with full-length IGF1 [109]. Recombinant IGF1 is available as a clinical formulation and is currently undergoing initial phase clinical trials in RTT patients (http://clinicaltrials.gov/ct2/show/record/NCT01253317?term=rett+syndrome).
biopsies from several brain regions, including cortex, basal ganglia and thalamus [110,111]. Deficits in bioamine levels also accompany the appearance of breathing abnormalities in MeCP2-null mice, which also show reduced levels of the primary synthesizing enzyme tyrosine hydroxylase in the brain as well as peripheral neurons and the adrenal medulla [112–114]. Monoamines are important regulators of brain stem function and therefore the antidepressant desipramine, which boosts noradrenaline signalling by blocking its uptake, has been tested in MeCP2-null mice. These studies reveal a delayed genesis of breathing abnormalities in young animals and a substantial amelioration in breathing abnormalities (apnoeas) when administered to mice that were already symptomatic [115,116]. At a cellular level, in vitro studies show irregular rhythms within brain stem networks isolated from MeCP2-null mice, but that application of noradrenaline can stabilize respiratory network rhythmogenesis [112]. However, although desipramine treatment improved respiratory function and extended the lifespan of MeCP2-null mice (from a modest increase up to double), it failed to alter growth curves or, surprisingly, levels of noradrenaline [116].

ACh (acetylcholine) is another neuromodulator, produced in midbrain nuclei, that shows alterations in the Rett brain. Specifically, several reports suggest impaired cholinergic function, such as a reduction in the synthesizing enzyme, choline acetyltransferase, and vesamicol binding to the vesicular transporter [117]. The actions of cholinergic ligands or acetylcholinesterase inhibitors have not been investigated to date, but the observed cholinergic dysfunction in the RTT brain led to the proposal that a diet enriched in choline, considered to be the primary synthesizing enzyme tyrosine hydroxylase in the brain. Specifically, several reports suggest impaired cholinergic function, such as a reduction in the synthesizing enzyme, choline acetyltransferase, and vesamicol binding to the vesicular transporter [117]. The actions of cholinergic ligands or acetylcholinesterase inhibitors have not been investigated to date, but the observed cholinergic dysfunction in the RTT brain led to the proposal that a diet enriched in choline, considered to be the primary constituent, might boost cholinergic function and be beneficial in RTT. This concept has been tested experimentally in MeCP2-null mice that were provided with choline during early postnatal development by delivery through maternal milk [118]. The results of this study were a modest improvement in certain locomotor and motor tasks, but the effects were very subtle and the treatment did not alter contextual or cued fear learning and was not reported to alter ultimate disease progression or survival.

Neurotransmitter systems: glutamate

Glutamate is the primary excitatory transmitter in the CNS and, as indicated above, loss of MeCP2 is widely reported to affect the function and structure of glutamatergic synapses. One of the most consistent findings in the MeCP2-null brain is a reduction in various forms of short- and long-term synaptic plasticity. In addition to neuron-to-neuron signalling, the neurotoxic actions of glutamate released from microglia (but not astrocytes) are also suggested to contribute to the onset and progression of RTT [119]. Thus, although some studies suggest loss of MeCP2 to be associated with a reduction in glutamatergic signalling, other reports suggest that excessive or sustained glutamate production, perhaps through non-neuronal sources, may contribute to the pathology. As indicated above, a positive modulator of the AMPA subtype of glutamate receptor, CX546, has been shown to improve various aspects of the breathing phenotype in the MeCP2-mutant mouse. Whether this is due to its boosting basal glutamatergic synaptic transmission, its restoring normal synaptic plasticity or its promotion of neuronal discharges and consequent elevation of BDNF and other activity-dependent trophic factors remains to be tested. The other major class of glutamate receptor is the NMDA (N-methyl-D-aspartate) receptor subtype. Mice lacking MeCP2 show altered NMDA subunit distribution within glutamatergic synapses [120]. NMDA receptors are at the heart of many forms of synaptic plasticity and are also important in excitotoxic processes. Memantine is a weak non-competitive antagonist of the NMDA receptor and is used in Alzheimer’s disease therapy by virtue of its proposed antagonism of sustained pathological activation of NMDA receptors and promotion of synaptic plasticity through reducing synaptic noise [121]. In a recent report, Weng et al. [45] showed that the progressive reduction in both short- and long-term forms of synaptic plasticity seen in MeCP2-mutant hippocampus can be partially reversed ex vivo using clinically relevant concentrations of memantine. Moreover, this treatment partially reversed plasticity deficits in the tissues from MeCP2-mutant mice, but did not affect glutamatergic synaptic transmission or plasticity in control mice. However, when applied in vivo, memantine failed to show any improvement in the RTT-like phenotype (tremor, abnormal breathing and reduced survival), suggesting that the improvement seen at the synaptic level is not reflected when considering the overall phenotype. However, it is possible that memantine may be useful in targeting other aspects of RTT such as cognitive impairment.

Neurotransmitter systems: GABA

GABA is the major inhibitory neurotransmitter in the brain, and selective silencing of MeCP2 in mice has revealed MeCP2 to be critical for the normal functioning of GABA-releasing neurons [122,123]. Dysfunction of GABA signalling is suggested to mediate some of the autism-like behaviours as well as other RTT-like phenotypes, including altered locomotor activity, motor function and breathing patterns [123]. Again, pharmacological studies aimed at modulating GABAAergic signalling in the MeCP2-mutant mouse have focused on the respiratory phenotype. Voiturou and Hilaire [124] used the fast-acting benzodiazepine midazolam (a positive allosteric modulator of several common GABA<sub>α</sub> receptor subtypes) to test the possible involvement of GABA receptor signalling in MeCP2-related breathing abnormalities. They found that the occurrence of apnoeas (after hypoxic or hypercapnic exposure) [125] and spontaneous prolonged inspiration events [126] were reduced, albeit transiently, in the MeCP2-null mouse by midazolam pretreatment. In another study, Abdala et al. [127] have shown that augmenting GABA, using a GABA-reuptake blocker, markedly improves the respiratory phenotype. The same authors have shown that a serotonin 1a receptor agonist (that depresses respiratory neuron activity) reduces apnoeas, corrects the irregular breathing and prolongs survival in MeCP2-null male mice and that a combination approach (GABA-reuptake blocker with a serotonin 1a agonist) completely corrects the respiratory defects in female MeCP2-mutant mice. It is clear that drugs targeting GABAergic neurotransmission may be of important future therapeutic benefit.

Drugs targeting MECP2 mutations

In addition to targeting downstream systems that are dysregulated in the absence of functional MeCP2, another pharmacological approach would be to target, further upstream, the immediate consequences of the specific mutation responsible for the MeCP2 deficiency in the particular patient. Many patients carry nonsense mutations in MECP2 (e.g. p.R168X, p.R255X and p.R270X), which are associated with PSCs (premature stop codons). Agents, such as aminoglycoside antibiotics (e.g. gentamycin), that permit ribosomal readthrough of PSCs during translation, would enable production of full-length functional protein [128,129]. Gentamycin binds a specific site on the ribosome and impairs...
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Codon/anticodon recognition at the tRNA acceptor site, thus bypassing the PSC by insertion of another amino acid (resulting in a missense mutation, often proving to be of little consequence) [130]. There are several examples where this concept has found clinical application. Malik et al. [131] showed that gentamycin application to boys with Duchenne muscular dystrophy for up to 6 months resulted in increased levels of dystrophin and decreased levels of serum creatine kinase. A similar strategy is also being employed for cystic fibrosis, where gentamycin enabled read-through of a PSC in the mRNA of the CTR (cystic fibrosis transmembrane conductance regulator) gene to produce full-length functioning protein in vitro [132,133].

On the basis of these previous studies and considering the fact that up to ~40% of typical RTT patients with MECP2 mutations have one of the nonsense mutations [134], aminoglycosides seem a promising avenue by which to achieve a restoration of full-length functional MeCP2. Brendel et al. [135] expressed four different common nonsense mutations (R168X, R255X, R270X, R294X) in HeLa cells and then tested the expression of full-length MeCP2 before and after addition of gentamycin. Full-length MeCP2 was detectable after gentamycin treatment, with variable expression efficiency (up to ~30%) depending on the type of mutation and the effective concentration of gentamycin used. In similar studies, gentamycin or geneticin were effective in producing full-length MeCP2 from R168X, R255X and R294X mutations (each having TGA codons) with the efficiency varying between drug and mutation (highest with geneticin and the R294X mutation respectively) [136]. In further work, amikacin and paromomycin were found to be ineffective in overcoming R168X and R255X mutations. Overall, these studies demonstrate a proof-of-concept for ribosomal readthrough of MECP2 mutations, but show that the readthrough efficiency varies enormously according to the type of drug, the type of mutation and the context of the stop codon [137]. Coupled with their toxic effects, these findings make it clear that currently available and approved aminoglycoside drugs are unlikely to form the core of a new therapeutic suite. A new generation of related compounds, typified by ataluren (also known as PTC124) could be more promising. Ataluren promotes efficient readthrough of UGA PSCs, is orally active, and is showing signs of being considerably less toxic than the structurally unrelated aminoglycosides [138]. Another aminoglycoside compound with reduced toxicity, NB54, is sevenfold more efficient than gentamycin [139]. However, little is known about the ability of PTC124 or NB54 to cross the BBB and so their likely efficiency in treating CNS disorders including RTT is unknown at present.

ENVIRONMENTAL AND EPigenetic FACTORS

If the critical role of MeCP2 disrupted in RTT is connected with its role in epigenetic regulation of transcription and downstream processing events, then tackling RTT by targeting therapeutic interventions to epigenetic processes might warrant attention. In addition, environmental approaches should not be discounted as promising avenues for investigation, especially where they potentially interact with epigenetic regulation processes. Clinical studies suggest that modification of the environment may have useful application in the management of children with RTT [140], and the influence of environment on the onset and progression of RTT-like features has been assessed systematically in Mecp2-null mice. Exposure to an enriched and stimulating environment can delay the onset and progression of neurological signs in a range of disease models, including models of Huntington’s disease [141] and Alzheimer’s disease [142]. These studies have utilized EE (environmental enrichment) in the form of increased social stimulation through group housing and increasing the complexity of housing through additional nesting materials, tunnels and other objects [143] which promote sensory, cognitive and motor stimulation relative to standard housing conditions. Kondo et al. [144] investigated the role of EE on the phenotype progression in Mecp2-null mice. The authors showed that post-weaning EE significantly reduced motor co-ordination defects and produced a modest increase in cerebellar BDNF levels in Mecp2−/− heterozygous females, but no change in locomotor function or brain BDNF levels in male Mecp2-null mice. In another study, Nag et al. [145] reported post-weaning EE to produce a similar amelioration of motor co-ordination as well as an improvement in motor learning. In contrast with the study by Kondo et al. [144], these authors also reported an improvement in locomotor activity and contextual fear conditioning (a hippocampus-dependent memory task) in Mecp2-null male mice [145]. Another study by Lonetti et al. [146] reported a similar improvement in motor co-ordination as well as motor learning in Mecp2-null males.

EE is known to result in a number of morphological changes in the brain (increased cortical thickness, dendritic branching and spine density) and promotes neurogenesis and the production of various neurotrophic factors [147]. At the molecular level, EE is also known to alter the expression of a large number of genes [148] and brain proteins [149]. Although the exact mechanism by which EE ameliorates some aspects of the RTT-like phenotype is unclear, these results nevertheless suggest that epigenetic modulators may be a possible route to therapeutic intervention. A recent report demonstrated that RTT-like anxiety phenotypes result from lack of functional MeCP2 (and presumed ensuing impaired transcriptional repression) within the basolateral amygdala and that these effects can be mirrored by local administration of a histone deacetylase inhibitor [150]. The development of drugs acting on epigenetic processes is a booming area of applied research and it is possible that future compounds may be found which specifically target aberrant transcriptional repression and be useful in treating RTT close to the upstream end of the pathogenetic process.

CONCLUDING REMARKS

Significant progress has been made in reversing symptoms and deficits in animal models of RTT and other related ‘neurodevelopmental’ disorders. The ability to rescue phenotypes in the adult brain that were previously thought to be due to aberrant development raises the question of whether such conditions are strictly ‘neurodevelopmental’ or whether they should rather be considered disorders of neuroplasticity or neuromaintenance [64]. This is not a purely semantic issue, but is of fundamental significance when considering the tractability of such conditions and the quest for future therapies. Recent years have seen a shift from basic studies aimed at identifying the molecular basis of RTT through to practical investigations of therapeutic intervention strategies. Although the unequivocal identification of all MeCP2 functions has remained elusive, the coming years are likely to see significant advances as new therapeutic avenues are identified and the genetic and pharmacological strategies discussed in the present review are formally tested.

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REFERENCES


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120 Frankiewicz and Parsons, C. G. (1999) Memantine restores long term potentiation impaired by tonic N-methyl-o-aspartate (NMDA) receptor activation following reduction of Mg^2+ in hippocampal slices. Neuropharmacology 38, 1253–1259


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