4. Discussion

4.1 MBD protein family

4.1.1 Expression of new MBD protein family members

For many years, MECP2 was thought to be a global transcriptional repressor. This assumption was based on *MECP2* expression in almost all tissues studied (D'Esposito *et al*., 1996, Coy *et al*., 1999) and the fact that MECP2 is essential for embryonic development in mice (Tate *et al.*, 1996). However, mutations in *MECP2* lead to Rett syndrome with an exclusively neuronal phenotype. This could be explained by a greater need of long lived, non-dividing neuronal cells for a special chromatin state that involves MECP2 and tightly suppresses transcription of genes not needed in that tissue. Another explanation would be that the loss of function of MECP2 in non-neural tissues is compensated by another protein with similar properties.

In this study, six proteins (BAZ2A, BAZ2B, KIAA1461/MBD5, KIAA1887/MBD6, SETDB1, and SETDB2) with a methyl-CpG-binding domain in mouse and man were identified as new members of the MBD protein family. Transcripts of *SETDB1, SETDB2, BAZ2A, KIAA1461/MBD5,* and *KIAA1887/MBD6* are found in all adult tissues studied. *SETDB2*, *BAZ2B*, and *KIAA1461/MBD5* show a low expression in brain and the mRNA levels of *KIAA1887/MBD6* are particularly lower than in other tissues. According to RT-PCR data from the HUGE database, *KIAA1461/MBD5* expression is basically confined to the cerebellum and the corpus callosum. The exact cell types that express *BAZ2A/TIP5, SETDB1,* and *SETDB2* as well as *KIAA1461/MBD5* and *KIAA1887/MBD6* in the brain are not known. MECP2 is found preferentially in mature neurons of the brain (Shahbazian *et al.*, 2002, LaSalle *et al.*, 2001). Should one of the other MBD proteins compensate for the loss of function of MECP2 in Rett syndrome it would be expected that the compensating gene shows an expression pattern complementary to that of *MECP2*, in particular in peripheral tissues that are not obviously affected in the disease. More detailed studies will be needed on the expression patterns of the MBD protein family members to confirm this hypothesis.

Based on gene expression studies with *Mecp2* knockout mice and on biochemical evidence, it has been suggested that the essential function of MECP2 in the brain might not be transcriptional regulation (Tudor *et al.*, 2002). In view of this aspect and of the protein–

protein interaction property of the methyl-CpG-binding domain of human MBD3 and SETDB1, functional compensation would not necessarily require a DNA binding capacity.
Except for KIAA1887/MBD6 all presented polypeptides are known or predicted to be involved in mechanisms of gene expression regulation. In order to understand the higher-order interplay of MBD proteins and associated complexes, it will be a major task to identify interacting proteins as well as regulated targets. This will help to solve the question whether some of these proteins can functionally complement MECP2 in tissues other than the brain.

4.1.2 The MBD domain of the new family members

The sequence comparison of the MBD domains of all MBD proteins showed only few amino acids conserved in all proteins. This raises the question whether the newly identified MBD sequences do really bind m^5CpGs.

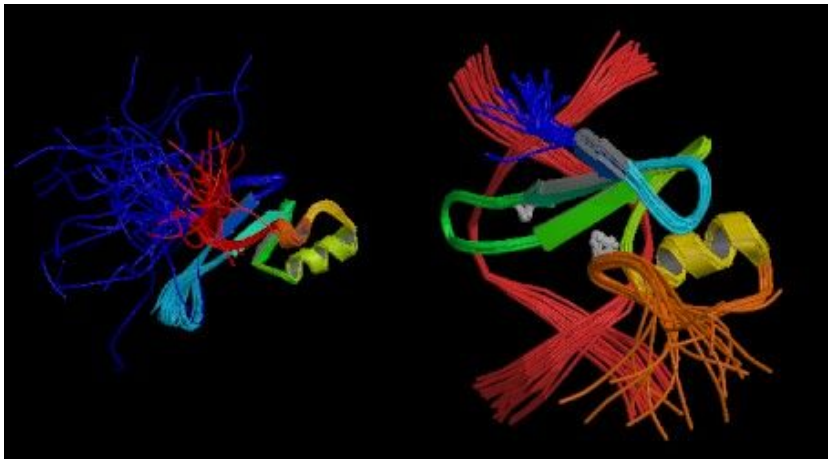

Fig. 25. Solution structure of the MBDs of MBD1 (left) and MECP2 (right). The α-helix (yellow) opposite the three β-sheets (arrows in green and blue), that form the 3D structure, can be seen. Loop 2, important for DNA binding, is turquoise in the MECP2 and green in the MBD1 structure. (Images adopted from PDB)

The solution structures of the methyl-CpG binding domains of three of the human MBD proteins, namely of MECP2 (Wakefield et al., 1999), MBD1 (Ohki *et al.*, 2001), and MBD4 (Wu *et al.*, 2003) have been published. A 3-D model of the MBDs of MECP2 and MBD1 are shown in Fig. 25. The structural data in these publications revealed residues important for DNA binding.
The following amino acids and structures have been found to be important for DNA and more specifically for m^5CpG binding of MECP2: Loop 2 is inserted in the major groove of the DNA while the α-helix interacts with the sugar-phosphate backbone. The methyl groups of the cytosines on the forward strand interact with hydrophobic side chains of Val-18, Tyr-32, and

Arg-20. The residues Arg-42 and Ser-43 interact with the methyl groups on reverse strand. Arg-20 and Arg-42 form hydrogen bonds with the guanines of the m^5CpG dinucleotides.

Amino acid	Residue present in
Val-18	MBD1, MBD2, and MBD3
Arg-20	MECP2, MBD1, MBD2, MBD3, MBD4, SETDB1, and BAZ2B
Tyr-32	MECP2, MBD1, MBD2, and MBD4
Arg-42	All MBD proteins except for MBD5, and MBD6
Ser-43	MECP2, MBD1, MBD2, MBD3, MBD4, and MBD6

Table 26. The existence of amino acids important for m^5CpG binding in MBD1 in other MBD protein family members. This summary shows that in the new MBD protein family members many of the residues are not conserved. Also, in MBD4 and MECP2 Val-18 is not present.

Important residues of the structure of MECP2 are summarized in Table 27. In contrast to the methyl-CpG binding domain of MBD1 where Val-18, Arg-20, Tyr-32, Arg-42, and Ser-43 are necessary for m^5CpG binding, in MECP2 also Ile-35 interacts with a methylated cytosine. This amino acid only exists in MECP2 and MBD4. Such structural differences probably cause the different binding specificities of the MBD proteins (see 1. Introduction).

The unrooted tree in Fig. 11 (Results 3.1.2) shows that the MBD domain of MECP2 is structurally most closely related to MBD4. However, MBD4 has a specificity for m^5CpG/GpT mismatches. This suggests, that either other domains present in the protein modify the binding specificity of the MBD domain or that minor changes in the MBD can lead to an altered binding behavior. Fig. 9 (Results 3.1.2) clearly shows that the methyl-CpG binding domains of all MBD proteins vary considerably in their polypeptide sequence. It is even more intriguing, that at least three of the proteins (MECP2, MBD1, and MBD2) specifically bind the very small m^5CpG motif. This suggests, that even a great variation in the sequence of the different MBDs still allows the same specialized m^5CpG binding function

Amino acid	Function in structure
Leu-9	Hydrophobic core
Trp-13	Hydrophobic core
Arg-15	Hydrophobic core
Leu-17	Hydrophobic core
Tyr-29	Hydrophobic core
Val-31	Hydrophobic core
Leu-33	Hydrophobic core
Phe-41	Hydrophobic core
Leu-47	Hydrophobic core
Phe-51	Hydrophobic core
Leu-59	Hydrophobic core
Phe-64	Hydrophobic core
Phe-66	Hydrophobic core
Val-68	Hydrophobic core
Tyr-33	Interaction with methylated C
Ile-35	Interaction with methylated C

Table 27. Residues of the MBD of MECP2 and their function. Amino acids of the hydrophobic core form the three β-sheets and the α-helix.

From the sequence alone it is not possible to predict whether the new MBD protein family members will bind m^5CpG or not. As far as the conservation of important residues in the Tables 26 and 27 are concerned, a binding is not very likely since many of the amino acids important for m^5CpG-binding of MBD1 are not conserved. To get certainty about the binding capabilities, DNA binding assays with the individual domain sequences will have to be carried out. Since the MBDs of MBD3 and SETDB1 have been shown to bind to other polypeptides, protein-protein interactions should also be studied (e.g. by yeast-two-hybrid screens).

This of course raises the question, whether the newly identified MBD proteins merit the name MBD. However, it is nowadays common to name proteins according to protein family members they show similarity to. A recent example are the proteins MBD3L1 and MBD3L2 that have homologies with MBD3 but do not even contain an MBD motif (Jiang *et al.*, 2002).

4.2 Search for MECP2 paralogues

A total of 17 proteins with an overall sequence similarity to MECP2 were found in the different searches applied. Only two proteins were detected in the SSDB as well as the BLink search, namely "myeloid/lymphoid or mixed-lineage leukemia (thrithorax homolog, Drosophila); translocated to, 2" (MLLT2) and "neurofilament, heavy polypeptide 200kDA" (NEFH). NEFH was detected in all three analyses.

Proteins that were found in the database queries but not in the BLAST search have their similarity probably due to regions of MECP2 with low complexity such as the histidine-rich region from aa 266 to aa 372 or the proline-rich region from aa 376 to aa 405 (Fig. 26). Polypeptides similar to each other only due to matches in these domains are not likely to be paralogues since such local resemblances will have arisen independently during evolution of the proteins and are not remains of the sequence of a common ancestor. Therefore only NEFH and MLLT2 were considered to have an overall sequence similarity to MECP2 since they were found in two database searches and NEFH is the most promising candidate as it was also detected in the BLAST search in which the low complexity regions were masked.

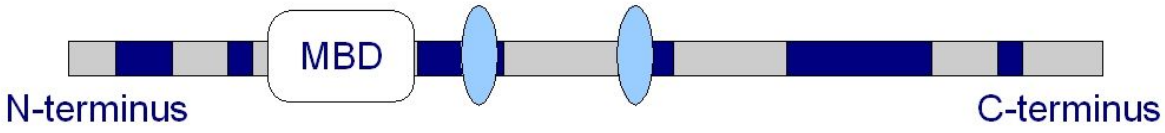

Fig. 26. Graphical representation of MECP2 with the low complexity regions depicted in blue. The MBD is shown in white, while the AT-hook motifs are indicated as turquoise ovals. The low complexity region next to the C-terminal AT-hook corresponds to the histidine-rich region, while the low complexity region further to the right is the proline-rich region in MECP2.

4.2.1 NEFH

NEFH is a member of the neurofilament protein family and is as such composed of a highly conserved alpha-helical core region of approximately 310 amino acids that form double stranded coiled-coils flanked by head (amino)- and tail (carboxy)-domains (Fig. 27).

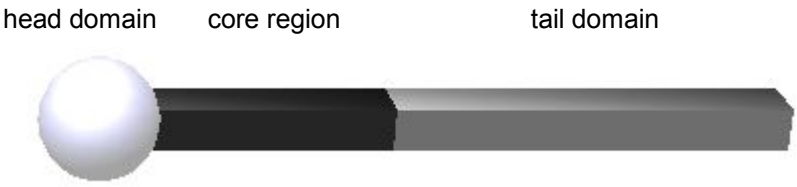

Fig. 27. Structure of the NEFH protein.. The C-terminal tail domain has sequence similarities to MECP2.

NEFH coassembles with "neurofilament, light polypeptide 68kDa" (NEFL) via its core region *in vivo* to form heteropolymers while it binds to neighboring filaments via the tail domain. The amino acid sequence lysine-serine-proline (KSP) is repeated 51 times in the central part of the tail domain. This aa triplet represents a major protein kinase recognition site (Julien *et al.*, 1998). This explains the high degree of phosphorylation of the protein and its large apparent M_r of 200 kDa, as determined by SDS-polyacryl amide gel electrophoresis (Liem *et al.*, 1978).

The tail region in particular has similarities to MECP2. The complete sequence alignment (Annex 6.1) shows a consensus for three KSP motifs between NEFH and MECP2, while the poly-histidine and poly-proline segments present in MECP2 do not occur in NEFH. Also the MBD domain of MECP2 at position 90-165 has few matching aa and two large gaps. Therefore, the KSP phosphorylation sites are the only motifs present in both sequences. Interestingly, the exact phosphorylation site of MECP2 as well as the kinase that adds the phosphate groups remain unknown. Based on this bioinformatics study, the KSP motifs represent candidate phosphorylation sites of MECP2.

Phosphorylation however, as the literature shows, has different effects on the two proteins. While MECP2 becomes inactivated by phosphorylation and consequently dissociates from the *Bdnf* promoter (Chen *et al.*, 2003), phosphorylation of NEFH has been shown to slow down filament transport in the axon (Ackerley *et al.*, 2003).

Finally, a recent publication (Klose and Bird, 2004) suggests, that MECP2 has an elongated shape which is supported by studies on its chicken homologue ARBP (von Kries *et al.*, 1994). This would correspond to the elongated form of NEFH.

4.2.2 MLLT2

There are currently no data on the structure of the *MLLT2* protein AF-4 in the literature. Also database searches did not result in any helpful hints. The only motif according to Pfam is the AF-4 motif (comprising the whole AF-4 protein) which is shared by FMR2. As yet, no

function has been assigned to this AF-4 domain.
Therefore no assumptions on the shape of MECP2 can be made with the help of AF-4. However, AF-4 has been reported to cause ataxia when mutated (Isaacs *et al.*, 2003), a symptom, that is also found in RTT.

4.3 MECP2 target genes

4.3.1 MECP2 regulates *Fkbp5* expression

Our microarray study revealed genes potentially regulated by MECP2 in the mouse brain. The expression of five of these differentially expressed genes was known to be regulated by glucocorticoids. Based on these results, two major questions arose: A) Are these genes direct targets of MECP2 or are the changes in gene expression due to secondary effects of loss of MECP2 function in the brain? B) How do glucocorticoids, known to act as transcription factors, interfere with transcription repression by MECP2?
To address these questions, chromatin immunoprecipitation was established, which allowed to analyze direct interactions of MECP2 with the genomic DNA of these potential targets. *Fkbp5* was chosen to be studied in further detail. Three MECP2 binding regions were revealed (region 1, region 2, and GRE_2), one of which (GRE_2) can also be bound by the GR. Addition of the glucocorticoid dexamethasone to the culture medium of primary neurons induced binding of GR to *Fkbp5* while binding of MECP2 was abolished. In contrast, the glucocorticoid inhibitor RU-486 reverses the effect of dexamethasone and binding of MECP2 to the GRE_2 region is re-established.
A model can therefore be proposed, in which GR and MECP2 control the expression of *Fkbp5* with glucocorticoids increasing and MECP2 decreasing the expression levels of *Fkbp5*. GR and MECP2 compete for the binding to at least on locus in the *Fkbp5* genomic region (i.e. GRE_2). If the concentration of either nuclear GR or MECP2 changes, the expression of *Fkbp5* will be altered (Fig. 28), which in turn leads to changes in GR signaling. This is due to the fact, that FKBP5 itself is involved in GR signaling (Davies *et al*., 2002). FKBP5 is bound to the GR receptor in the cytoplasm and replaced by FKBP4 after binding of GR to glucocorticoids. This then leads to translocation of the receptor complex into the nucleus and consequent gene expression regulation by GR.

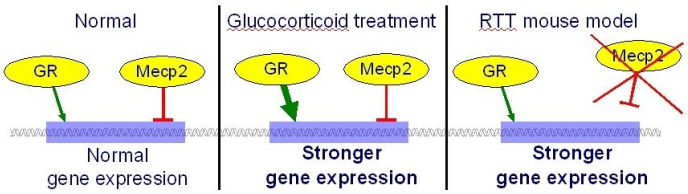

Fig. 28. Model of *Fkbp5* gene regulation. The GR and MECP2 regulate the same gene. Stronger activation due to higher GR levels after glucocorticoid treatment or missing repression due to loss of function of MECP2, both situations lead to a stronger expression of the target gene (blue bar).

It is noteworthy, that MECP2 not only has one binding site in the *Fkbp5* genomic region, but seems to regulate *Fkbp5* gene expression by binding to several loci. The first binding site is in the proximal promoter, the second one near the start of the second exon. The third binding site (GRE_2), however, is in the fifth intron, about 50 kb away from the core promoter. This raises the question how a binding far downstream of the transcription start can influence the expression of the gene. Nevertheless, this regulatory element probably is important since at least for the human *FKBP5* gene it has been shown that expression is regulated by GR via a binding site 2 kb downstream from the promoter (U *et al.*, 2004). In addition, the results of ChIPs with a GR antibody in this thesis prove that the GRE_2 region is a binding site for GR in mouse.

An antibody specific for the C-terminal part of MECP2 was used to perform ChIP. Therefore, binding of both MECP2 isoforms (MECP2e1 and MECP2e2) was detected simultaneously. In follow-up studies the use of antibodies specific for the N-terminal parts of MECP2e1 or MECP2e2 should allow to determine if the two variants bind to the same targets or have different specificities. Antibodies specific for an N-terminal antigen might, however, be difficult to use in chromatin immunoprecipitation since the antigen would be close to the MBD and therefore probably poorly accessible after binding to the DNA and formaldehyde treatment.

4.3.2 Northern blot and quantitative real-time PCR results support the microarray findings

Our collaborators in Edinburgh carried out experiments that substantiate our findings:

To confirm the results obtained by our microarray experiment, Northern blot analyses and

quantitative real-time PCRs were carried out in Edinburgh. Their Northern blot results show the differential expression of these transcripts in *wt* as compared to symptomatic *Mecp2*$^{-/y}$ mice.

For real-time PCR analysis, *Mecp2*-null mice were grouped into three categories: pre-symptomatic, early-symptomatic and late-symptomatic. Male *Mecp2*-null mice acquire neurological symptoms at ~ 6 weeks of age and die at ~ 10 weeks. The postnatal onset of symptoms resembles RTT, as the affected girls develop normal until the age of 6 – 18 months. This raised the question whether the de-regulated expression of the *Sgk* and *Fkbp5* genes seen in late symptomatic mice was also present in animals that had yet to develop symptoms. As there is considerable heterogeneity in the timing of symptom-onset and progression, *Mecp2*-null animals were classified according to symptoms rather than age using the criteria shown in Table 28.

Phenotype	Clasping	Inertia	Tremor	Weight loss	LOC*	Average age
Presymptomatic	-	-	-	-	-	~30d
Early symptomatic	-/+	+	+	-	-	~55d
Late symptomatic	+	+	+	+	+	~ 70d

Table 28. Classification of *Mecp2*-null mice according to the manifestation of phenotype. The last column shows the average age of mice displaying the symptoms. *LOC – loss of condition.

Real-time PCR, relative to *Gapd* as an internal control, revealed that *Sgk* and *Fkbp5* are both up-regulated in pre-symptomatic and early symptomatic mice in comparison to *wt* controls (Fig. 29B). Further analysis of the same cDNA samples established that many other genes show indistinguishable expression levels in mutant and *wt* mice at all three stages (data not shown) in agreement with previous results (Tudor *et al*., 2002). The de-regulation of *Sgk* and *Fkbp5* genes in mice with no obvious symptoms supports the possibility that they are direct targets for MECP2.

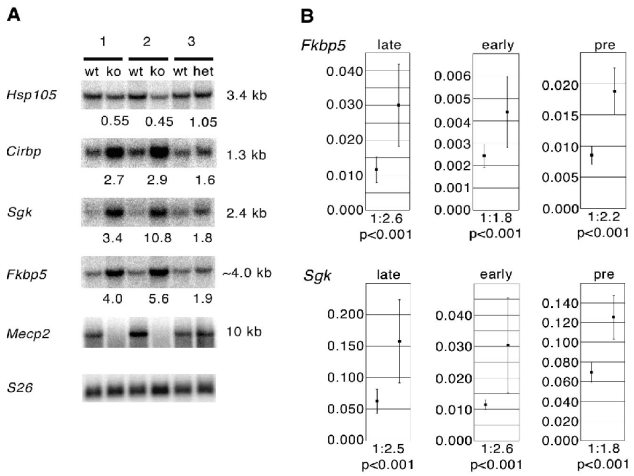

Fig. 29. Northern blot and real-time PCR results confirm microarray data. A) Northern blot analyzes of *Hsp105*, *Cirbp*, *Sgk* and *Fkbp5* confirm the microarray results for these genes. Total brain RNA isolated from *Mecp2*$^{-/y}$ (ko), heterozygote *Mecp2*$^{+/-}$ (het) and wild-type (*wt*) control animals was used. Three litter mate pairs (1, 2, 3) were investigated. The *Mecp2*$^{-/y}$ animals showed advanced neurological symptoms. Intensities of the *Hsp105*, *Cirp*, *Sgk*, and *Fkbp5* bands were normalized against a loading control (*S26 ribosomal protein* cDNA probe). Expression levels of *wt* animals were arbitrarily set to 1.0 and relative expression levels of the respective ko and het litter mate samples are indicated below the lanes. (B) Real time PCR analyzes of *Fkbp5* and *Sgk* in pre-symptomatic (pre) animals, and animals with early or late symptoms as defined in Table 28. Nine *wt* and nine *Mecp2*-null animals were investigated per stage. A significant up-regulation of both genes was detected in all three investigated stages (see p-values). The mean is shown as a dot; whiskers indicate ± the standard deviation. The y axes represent arbitrary threshold cycle number converted into expression levels relative to *Gapd* expression.

4.3.3 Up-regulation of glucocorticoid–inducible *Fkbp5* and *Sgk* in *Mecp2*-null animals is not due to elevation of glucocorticoid levels

As hyper-secretion of glucocorticoids has not been shown to induce expression of *Fkbp5* and *Sgk* in the brain, pumps were implanted into *wild-type* mice by Dr. Holmes in Edinburgh. These pumps delivered a corticosterone solution at a constant rate. The effect on transcription of *Fkbp5* and *Sgk* was measured. The experiments were performed by Northern analyses of the mRNA levels in the brains of cortisol treated mice and vehicle treated control animals and the results showed that two days of hormone exposure induced expression of both *Sgk* and *Fkbp5* compared to animals that received the solvent vehicle alone (Fig. 30 A). To determine whether the comparable level of induction of *Sgk* and *Fkbp5* in *Mecp2*-null mouse brain is due to high levels of circulating glucocorticoids, basal and stressed hormone levels were measured in Edinburgh. There was no significant difference between the basal plasma glucocorticoid levels of *wt* and *Mecp2*-null mice (Fig. 30 B), suggesting that elevated hormone levels are not responsible.

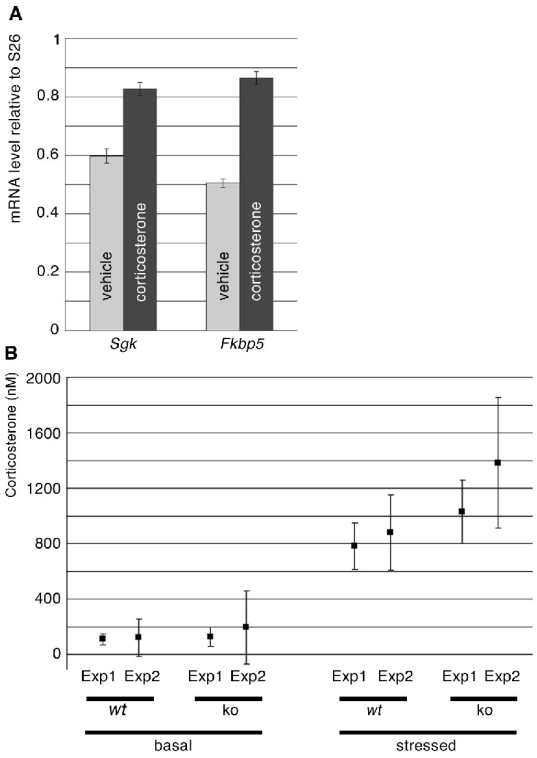

Fig. 30. Hormone-dependent and hormone-independent induction of *Fkbp5* and *Sgk* in mouse brain. (A) Corticosterone administration induces *Fkbp5* and *Sgk* expression in wt mouse brain. Hormone (n = 6) or hormone-free vehicle (n = 7) were delivered continuously to wt mice for 2 days, after which *Fkbp5* and *Sgk* mRNA levels in mouse brain were measured by Northern blotting. Results were normalized against S26 ribosomal protein mRNA levels on the same blots. (B) Corticosterone levels are not significantly elevated in *Mecp2*-null mice, although *Fkbp5* and *Sgk* are induced. Resting and stressed levels of plasma glucocorticoid were measured in symptomatic *Mecp2*-null mice (ko) and *wild-type* litter mates. Experiment 1 (n = 4) and experiment 2 (n = 5) are shown separately. Boxes depict mean plasma glucocorticoid values and whiskers show ± the standard deviation. Statistical analysis using Students t-test gave p-values for the comparison *wt* versus ko under basal conditions as 0.6673 (experiment 1 Exp1) and 0.5891 (Exp2). Under stressed conditions, p values were 0.141 (Exp1) and 0.1037 (Exp2). It can be concluded, that there are no significant differences between *wt* and ko hormone levels.

By ChIP analysis - carried out in Edinburgh - no changes in MECP2 binding to the regions fkp and fkp1 could be shown after implantation of corticosterone pumps (see Fig. 31). This suggests, that MECP2 and GR either regulate *Fkbp5* via independent sites or that there is another binding site of MECP2 that can also be bound by GR (see Fig. 23, 24). As shown by the experiments with glucocorticoid-treated primary neurons in this thesis, the GRE_2 region probably is such a binding site. This region has not been studied in mice, treated with corticosterone, so far.

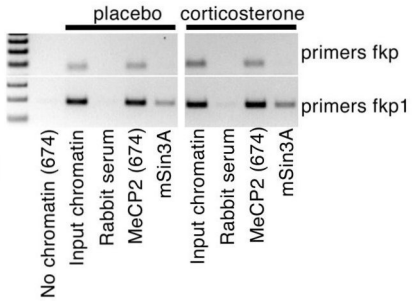

Fig. 31. MECP2 binding to the *Fkbp5* gene is not abolished in *wt* mouse brain upon corticosterone infusion as shown by ChIP with placebo and corticosterone-treated mice. Rabbit serum is used a negative control. mSin3A is a co-repressor complex interacting with MECP2 and appears to bind to the fkp1 site but not to the fkp region.

The data presented in the Results section and the results obtained by our collaborators in Edinburgh support the suggestion that certain downstream consequences of the glucocorticoid pathway are constitutively activated in this mouse model, even though glucocorticoid levels are not increased. Instead, the activation seems to be due to the missing repressor function of MECP2.

The activation of genes that are physiologically induced by glucocorticoids is in line with the observation that mice with different mutations in the *Mecp2* gene reportedly display seizures and heightened anxiety that may be attributed to the inappropriate activation of stress response genes (Chen *et al.*, 2001; Shahbazian *et al.*, 2002).

4.3.4 Rett syndrome: a stress pathway disease - supporting evidence from the literature

Several pieces of evidence suggest that constitutive activation of glucocorticoid-responsive genes may be relevant to our understanding of human RTT. Cortisol levels in Rett syndrome patients appear normal (Echenne *et al.*, 1991; Huppke *et al.*, 2001), but the over-alertness and agitation that may result from hormone-independent activity of stress pathways, including prolonged fits of screaming and crying, are characteristic features of RTT.

Elevated glucocorticoid exposure has profound, deleterious effects on the development of the fetus and neonate, altering cell proliferation, migration, and differentiation (de Kloet *et al.*, 1988). Early postnatal administration of glucocorticoids has been shown to result in an adverse outcome at school age with some similarities to Rett syndrome. Affected children show significantly reduced height and head circumference, poor motor skills and motor coordination, and significantly lower IQ scores (Yeh *et al.*, 2004). Prenatal glucocorticoid exposure also increases anxiety behavior in adult offspring (Welberg *et al.*, 2001) and postnatal exposure alters cerebellar development and function in rodents (Bohn *et al.*, 1980). The decreased dendritic branching and shortened dendrite arbors that have been described in Rett syndrome patients (Armstrong, 2002) are also mimicked by chronic administration of glucocorticoids, leading to dendritic atrophy of hippocampal neurons and dendritic reorganization of prefrontal cortical neurons (Wellman, 2001). In addition, osteopenia frequently occurs in Rett syndrome patients and is a known side-effect of glucocorticoid exposure (Haas *et al.*, 1997; Leonard *et al.*, 1999; Budden and Gunness, 2001).

In summary, many of the phenotypic features of Rett syndrome can be explained by altered expression of the glucocortoid-regulated genes. Of course *Fkbp5* will not be the only gene

regulated by MECP2 that contributes to the phenotype of the Rett mouse model we used. Rather the sum of many expression changes will constitute the disease.

4.3.5 Repression by MECP2: global and total versus local and partial

The results of the Northern blot, RT-PCR, immunostainings, and ChIP experiments for *Fkbp5* show, that MECP2 does not regulate *Fkbp5* in an "all-or-none" manner, but is rather responsible for the fine tuning of *Fkbp5* expression. Binding of MECP2 therefore might not condense the chromatin completely, but result in a chromatin state that makes transcription more difficult. Such an attenuating function of MECP2 has already been shown for the *Bdnf* gene (Chen *et al.*, 2003, Martinowich *et al.*, 2003).

If this model holds true for many other MECP2 targets, this would explain the difficulties scientists have had with microarray studies for Rett syndrome. An attenuating function of MECP2 would lead to small changes in gene expression which are hard to measure by microarray experiments. Tudor and colleagues could only detect subtle transcriptional changes using different brain samples (Tudor *et al.*, 2002) and the number of differentially expressed genes found in this study is much lower than what would be expected for a transcription factor with an extremely abundant binding site such as MECP2.

4.3.6 DNA microarray studies and chromatin immunoprecipitation

The results of this thesis show, that the combination of DNA microarray analysis and chromatin immunoprecipitation are extremely useful tools to find target genes of transcription factors as well as the corresponding binding sites. The disadvantage is, that both techniques are not trivial require laborious optimization. Some critical factors for chromatin immunoprecipitation have been outlined in the results part.

This study also highlights, that the appropriate biological material has to be chosen to obtain meaningful results. Only by using RNA from brain tissue of severely symptomatic *Mecp2*$^{-/y}$ mice for the microarray study and appropriate brain tissue as well as primary neurons for the ChIP, *Fkbp5* could be detected as a direct target of MECP2. Microarray studies carried out with primary fibroblasts, lymphoblastoid cells and postmortem human brain did not find *Fkbp5* to be differentially expressed. The binding pattern of MECP2 in fibroblasts and lymphoblastoid cells might be totally different from that in the brain. Even the more so if MECP2 only has an attenuating function.

In that perspective it would be interesting to hybridize the DNA fragments obtained by chromatin immunoprecipitation to a high-resolution genomic microarray covering the whole genome. This would allow to identify many more, possibly all MECP2 targets in one single experiment and enable a comparison of its binding pattern in different cell types and cellular states possible.

4.3.7 Future studies

This study suggests that enhanced glucocorticoid signaling occurs in RTT patients and may be therapeutically addressed by antagonists of the glucocorticoid pathway, e.g. at the GR level. To further analyze the interplay of MECP2 and the glucocorticoid receptor target genes, studies in animal models should be carried out in the future:

1. Administration of glucocorticoid pathway inhibitors to Rett syndrome animal models should reverse at least part of the phenotype.
2. If the activation of the glucocorticoid genes is due to loss of function of MECP2, a mouse GR-null mutant crossed to the *Mecp*$^{-/y}$ strain should reverse the phenotype.
3. It will be interesting to see at what time point in development the regulation of *Fkbp5* by MECP2 starts. The answer should shed some light on the course of the disease and how long it takes from the start of the dysregulation until the first symptoms appear.
4. Hybridization of the products of a ChIP with an anti-MECP2 antibody to a high-resolution whole-genome microarray would allow to find additional genes and pathways involved in the pathogenesis of Rett syndrome and might also resolve the questions why MECP2 mutations have a brain-specific phenotype.

4.4 Conclusions

Three aspects of MECP2, its function and its role in Rett syndrome have been studied in this thesis.

- Six new MBD family proteins with possible functional redundancy to MECP2 were found. Five of these proteins (SETDB1, SETDB2, BAZ2A, MBD5, and MBD6) are candidates to compensate for the loss of function of MECP2 outside the brain in Rett patients.
- Database analysis revealed homology of MECP2 to several polypeptides in the human proteome. The best matches were NF-H and MLLT2/AF4. A closer investigation of the homologous regions indicated, that overall MECP2 might have an elongated structure and revealed putative phosphorylation sites of MECP2.
- Finally, data presented in this thesis suggest, that downstream targets of the stress pathway might be involved in the pathology of Rett syndrome. More specifically, MECP2 regulates some genes that can be induced by glucocorticoids. Loss of function therefore leads to an increase in expression of these genes, which in turn may explain many features of the Rett syndrome phenotype. This gives rise to the hope, that inhibitors of the glucocorticoid pathway could constitute a treatment for Rett syndrome patients.