Biochemical and Biophysical Research Communications 437 (2013) 67–73

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Ube3a/E6AP is involved in a subset of MeCP2 functions

Soeun Kim [a], Maria Chahrour [b,1], Shay Ben-Shachar [b,2], Janghoo Lim [a,*]

[a] Department of Genetics, Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, CT 06510, USA
[b] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA

ARTICLE INFO

Article history:
Received 3 June 2013
Available online 19 June 2013

Keywords:
Rett syndrome
Angelman syndrome
Ube3a/E6AP
MeCP2

ABSTRACT

Rett syndrome (RTT) and Angelman syndrome (AS) are devastating neurological disorders that share many clinical features. The disease-causing mutations have been identified for both syndromes. Mutations in *Methyl-CpG Binding Protein 2* (*MECP2*) are found in a majority of patients with classical RTT while absence of maternal allele or intragenic mutation in the maternal copy of *UBE3A* gene encoding the human papilloma virus E6-associated protein (E6AP) cause most cases of AS. Extensive studies have been performed to determine the cause of the neurological problems in each disease. However, the genetic and molecular basis of the overlap in phenotypes between RTT and AS remains largely unknown. Here we present evidence that the phenotypic similarities between the two syndromes might be due to the shared molecular functions between MeCP2 and E6AP in gene expression. Our genetic and biochemical studies suggest that E6AP acts as an essential cofactor for a subset of MeCP2 functions. Specifically, decreased expression of *Ube3a* was able to rescue the cellular phenotypes induced by *MECP2*-overexpression in *Drosophila*. And biochemical assays using mice and cell culture systems show that MeCP2 and E6AP physically interact and regulate the expression of shared target genes. Together these data suggest that MeCP2 and E6AP play a role in the transcriptional control of common target gene expression and provide some insight into why RTT and AS share several neurological phenotypes.

1. Introduction

Rett syndrome (RTT) and Angelman syndrome (AS) are genetically distinct but phenotypically similar diseases with many shared clinical features including impaired social interactions, absence of speech, cognitive impairment, ataxia, and characteristic abnormal behaviors. Mutations in the gene encoding Methyl-CpG Binding Protein 2 (MeCP2) are found in the majority of patients with typical RTT [1]. Most cases of AS are caused by deletions in the maternal chromosome 15q12 containing the *UBE3A* gene, intragenic mutations in the maternal allele of the *UBE3A* gene, or absence of maternal chromosome 15 (with paternal uniparental disomy of the chromosome) [2–4]. *UBE3A* encodes the human papillomavirus E6-associated protein (E6AP) [5]. In addition to mutations and deletions, individuals with chromosomal duplications encompassing either the *MECP2* or *UBE3A* loci show clinical features related to those of RTT, AS, or autism spectrum disorders (ASDs) [6,7]. These data provide strong evidence that tight regulation of *MECP2* and *UBE3A* expression is essential for proper brain function and that mutations in either *MECP2* or *UBE3A*, or changes in the expression levels of their respective proteins, lead to neurological disorders.

Both MeCP2 and E6AP are multi-functional proteins. MeCP2 modulates RNA at several levels including transcription and alternative RNA splicing [8–11]. MeCP2 was originally found to function as a methylated CpG dependent transcriptional repressor [8] by associating with corepressor complexes containing Sin3A and Brahma and histone deacetylases, and/or CoREST [12,13]. In contrast, MeCP2 was shown to up-regulate gene expression by cooperating with transcription factor CREB1 [10]. Interestingly, several groups recently reported that MeCP2 plays a critical role in the proper organization of chromatin structures [14–16]. E6AP is an E3 ubiquitin ligase [5], but it also acts as a transcriptional coactivator for several proteins including the nuclear hormone receptors [17].

The fact that RTT and AS share many clinical features raises the question of whether they impact similar pathways. It is becoming abundantly clear that proteins that function in the same or interconnected molecular pathways cause similar phenotypes. For example, proteins implicated in human inherited ataxias, a genetically heterogeneous but clinically similar group of disorders, do indeed interact either directly or indirectly [18]. We rationalized that this may also be the case for RTT and AS, given the overlapping neurological phenotypes in both disorders. In this study we set out to test whether alterations in *Ube3a* expression can modulate the phenotypes of *MECP2* overexpression using a combination of

* Corresponding author. Fax: +1 203 785 5098.
E-mail address: janghoo.lim@yale.edu (J. Lim).
[1] Current address: Division of Genetics, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA.
[2] Current address: Genetic Institute, Tel-Aviv Sourasky Medical Center, Tel-Aviv, Israel.

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.06.036

genetic and biochemical approaches and whether any *in vivo* interactions are due to some shared or overlapping molecular functions of MeCP2 and E6AP.

2. Materials and methods

2.1. Creation of MECP2-GFP transgenic mouse line and mouse husbandry

Using BAC/PAC recombineering technology [19], a PAC DNA clone (PAC671D9) containing the human *MECP2* gene was modified by inserting the coding sequence for the Enhanced Green Fluorescent Protein (EGFP) downstream of the *MECP2* coding sequence to generate the *MECP2-GFP* clone. Linearized DNA containing the human *MECP2-GFP* fusion gene was purified and injected into single cell zygotes of FVB mice. Several *MECP2-GFP*^{Tg} lines were established and MeCP2-GFP expression was analyzed. *MECP2-GFP*$^{Tg/+}$ and *MECP2*$^{Tg1/+}$ [20] animals were maintained on a pure FVB/N background. *Ube3a*$^{m+/p-}$ [21] animals were maintained on a pure 129S6/SvEv background. *MECP2*$^{Tg1/+}$ male mice were bred to *Ube3a*$^{m+/p-}$ heterozygote females and all four subsequent F1 progeny were obtained. All mice were maintained on a 12 h light and 12 h dark cycle with standard mouse chow and water *ad libitum*. All research and animal care procedures were approved by the Baylor College of Medicine and the Yale University Institutional Animal Care and Use Committee.

2.2. Western blot analysis

Samples were prepared from adult fly head or whole brain or specific brain regions of mice, collected into 1 ml lysis buffer (50 mM Tris [pH 7.5], 150 mM NaCl, 1% SDS, 1% Triton X-100, 1 mM PMSF and Roche complete protease inhibitor cocktail) and briefly homogenized. The lysate was rotated at 4 °C for 20 min and then centrifuged for 15 min at 13,000 rpm at 4 °C. The supernatant was quantified and 20 or 40 μg total protein from each sample was analyzed by western blot. The following primary antibodies were used: mouse anti-tubulin (clone 12G10, Developmental Studies Hybridoma Bank), mouse anti-Gapdh (Advanced Immunochemical), mouse anti-HA clone HA-7 (Sigma–Aldrich), mouse anti-MeCP2 (Sigma–Aldrich), mouse anti-E6AP (Sigma–Aldrich), rabbit anti-GFP (Abcam), rabbit anti-Sst (Santa Cruz Biotechnology), rabbit anti-CREB (clone 48H2, Cell Signaling Technology), rabbit anti-E6AP (Bethyl Laboratory), rabbit anti-MeCP2 (Millipore), rabbit anti-mSin3A (clone AK-11, Santa Cruz Biotechnology), and rabbit anti-CoREST [13].

2.3. Co-immunoprecipitation assays

To investigate the interaction between MeCP2 and E6AP, co-immunoprecipitation experiments were performed using HEK293T cell extracts as similarly described [22]. HA-E6AP was a gift of Dr. Howley (Addgene plasmids, #8657, 8658, 8659) [23]. To test the MeCP2-E6AP interaction *in vivo*, whole brain of *MECP2-GFP*$^{Tg/+}$ (or *MECP2-GFP*$^{Tg/+}$; *Ube3a*$^{m-/p+}$) transgenic mice was dissected, collected into 2 ml hypotonic buffer (10 mM HEPES [pH 7.4], 1.5 mM $MgCl_2$, 10 mM KCl, 0.2 mM PMSF, 0.5 mM DTT, and protease inhibitor cocktail) and homogenized. Samples were rotated at 4 °C for 20 min and then centrifuged for 15 min at 1600 rpm at 4 °C. Pellets were washed at least twice with the same hypotonic buffer. Pellets were then lysed in lysis buffer (0.5% Triton X-100, 20 mM Tris [pH 8.0], 100 mM NaCl, 1 mM EDTA, 1 mM PMSF, and protease inhibitor cocktail) for 15 min at 4 °C, rotating, and soluble protein extracts were collected after centrifugation for 15 min at 13,000 rpm at 4 °C. Anti-E6AP, anti-GFP or normal rabbit IgG were used for immunoprecipitation. The immunocomplexes were washed three times with the same lysis buffer, and analyzed by SDS–PAGE and western blot.

2.4. Luciferase reporter assay

HEK293T cells were transfected using Lipofectamine 2000 with the pGL3-Basic-Sst-promoter luciferase reporter construct (50 ng) [10], the pRL-TK vector (10 ng) as an internal transfection control, pCDNA3.1-MECP2 (100 ng) and HA-E6AP (100 ng) vectors. Total plasmid amounts were adjusted by adding empty plasmid. After approximately 1 day later, cells were lysed and subjected to a dual luciferase reporter assay (Promega). Normalized luciferase activity was obtained by dividing the *firefly* luciferase activity from the reporter construct by the *Renilla* luciferase activity from the pRL-TK vector. All experiments were performed in triplicate.

2.5. Quantitative real-time PCR

The RNA extraction, cDNA synthesis, and quantitative real-time PCR were performed as described [10].

2.6. Statistical analysis

Data were analyzed using *ANOVA* (analysis of variance) using SPSS 18 software for Mac OSX or Student's *t*-test (two-tailed, not assuming equal variances).

2.7. Drosophila strains and scanning electron microscopy

The mutant and transgenic flies used in this study are *dUbe3a* (alleles 6J, 80, 15b) [24] and *UAS-MECP2* [25]. Processing and image acquisition of *Drosophila* eyes for scanning electron microscopy were performed by the EM Facility CCMI at Yale University.

3. Results

3.1. Decreased expression of Ube3a rescues MECP2-overexpression phenotypes in Drosophila

To explore the possibility that the neurological phenotypes associated with altered MeCP2 or E6AP levels may be mediated by some shared molecular functions between the two proteins, we performed a genetic interaction study using *Drosophila* models of *MECP2* and *Ube3a*. Overexpression of the human *MECP2* gene in the *Drosophila* eye causes disruption in the structured pattern of the normal eye surface [25]. Similarly, overexpression of *Ube3a* causes many abnormal phenotypes in *Drosophila*, while loss of *Ube3a* expression does not produce any detectable alterations in the *Drosophila* eye [24]. Therefore, these models provide an excellent opportunity to determine if loss or decreased expression of *Ube3a* can modify *MECP2* overexpression phenotypes. We looked for a genetic interaction between *MECP2* and *Ube3a* by crossing *MECP2* overexpression flies with *Drosophila Ube3a* (*dUbe3a*) mutant flies. A heterozygous loss of a single *dUbe3a* allele strongly suppressed ommatidial disorganization phenotypes, without affecting MECP2 expression, in flies expressing human *MECP2* (Fig. 1). These genetic interaction data suggest that *Ube3a* has a crucial role in inducing or mediating *MECP2*-induced abnormal phenotypes in *Drosophila*.

3.2. E6AP and MeCP2 do not regulate each other's expression

We next investigated the molecular relationship between MeCP2 and E6AP. Since reduced expression of E6AP did not affect

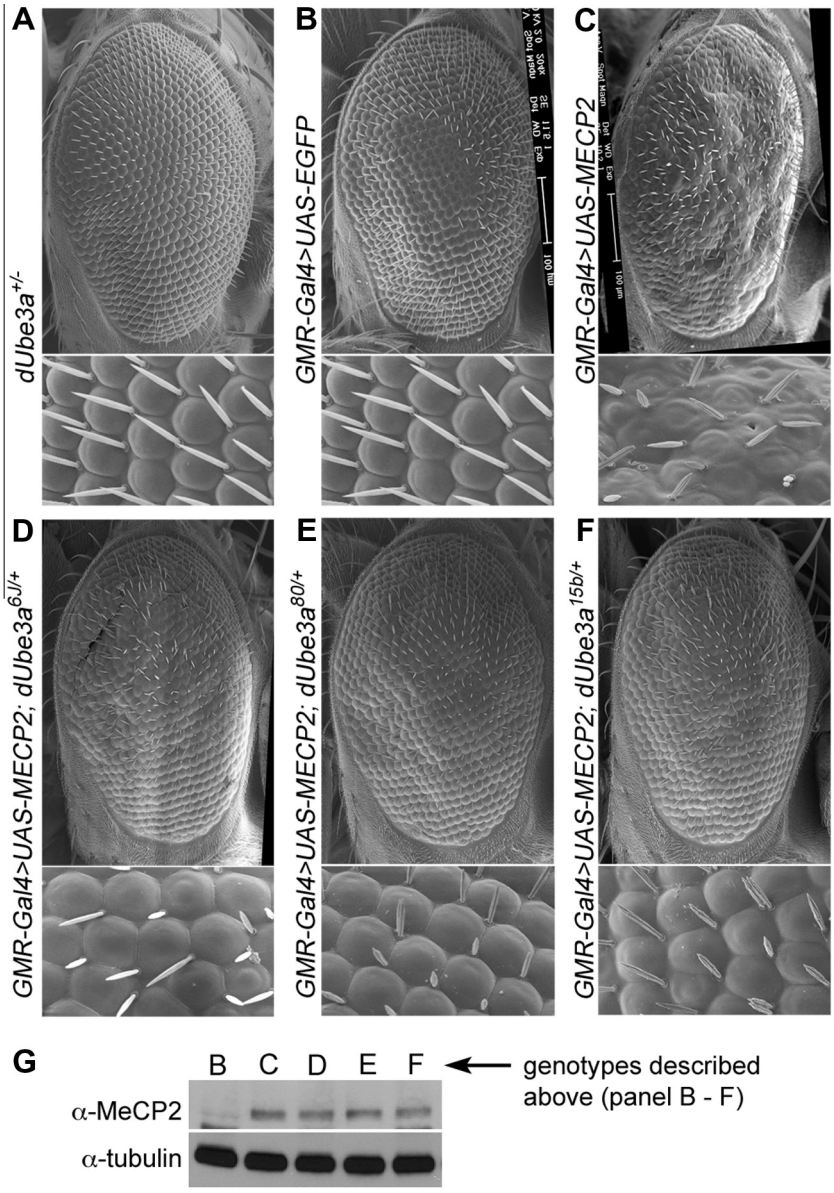

Fig. 1. Decreased expression of *dUbe3a* suppresses *MECP2* overexpression phenotypes in *Drosophila*. (A–F) Scanning electron microscopy of adult fly eyes from controls (A, B) or animals expressing human *MECP2* driven by the *GMR-Gal4* driver (C–F). Loss of one *dUbe3a* allele robustly suppressed the *MECP2*-mediated retinal disorganization phenotypes (D–F). Magnified images are at the bottom of each panel. More than 100 adult flies per genotype were examined at day 2 after eclosion. Three independent mutant alleles (called 6J, 80, and 15b) of *dUbe3a* showed the same results. (G) Western blot with a specific MeCP2 antibody in extracts from MECP2 expressing adult fly head shows that decreased expression of *dUbe3a* (50% reduction) did not affect *MECP2* protein expression. Flies were raised at 28 °C and genotypes are: (A) $dUbe3a^{+/-}$, (B) *GMR-Gal4/UAS-EGFP*, (C) *GMR-Gal4/UAS-MECP2*, (D) *GMR-Gal4/UAS-MECP2*; $dUbe3a^{6J/+}$, (E) *GMR-Gal4/UAS-MECP2*; $dUbe3a^{80/+}$, and (F) *GMR-Gal4/UAS-MECP2*; $dUbe3a^{15b/+}$.

MECP2 expression in *Drosophila* (Fig. 1G), we tested whether this is also the case in mice. We checked MeCP2 protein levels in the whole brain or specific brain regions of *Ube3a*-null mice and found no obvious difference in the MeCP2 protein level (Fig. 2A; data not shown). Conversely, we also examined the level of E6AP in mice with altered MeCP2 levels. E6AP expression was not altered in whole brain extracts of *MECP2* transgenic (*MECP2*-TG) (Fig. 2B) or *Mecp2*-null male mice (Fig. 2C) or in specific brain regions (data not shown). Taken together, these analyses suggest that E6AP and MeCP2 do not affect each other's expression.

3.3. E6AP cooperates with MeCP2 to regulate target gene expression

We next asked whether MeCP2 and E6AP could function together in regulating target gene expression, given that MeCP2 and E6AP both act as regulators of gene expression independently [10,17,26,27]. Since E6AP can act as a transcriptional coactivator [17], we tested whether E6AP acts as a coactivator for MeCP2. We transfected HEK293T cells with a luciferase reporter construct driven by the promoter of *Somatostatin* (*Sst*), a known transcriptional target activated by MeCP2 [10]. Co-expression of MeCP2 with E6AP resulted in significant enhancement of Sst reporter activity (Fig. 3A). E6AP has three isoforms that differ at their N-terminus. Interestingly all three isoforms of E6AP could enhance the MeCP2-mediated Sst luciferase activity (Fig. 3A), suggesting that E6AP cooperates with MeCP2 to enhance transcription, at least in this cell culture system.

To ascertain the relevance of E6AP activity in MeCP2-mediated gene expression *in vivo*, we investigated whether the expression levels of activated MeCP2 targets are affected by the presence or

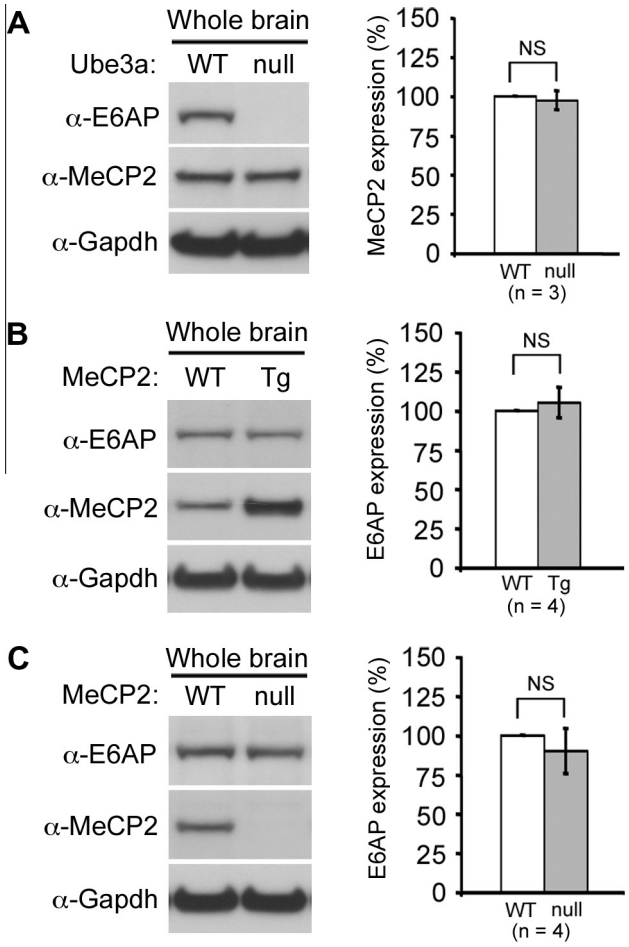

Fig. 2. MeCP2 and E6AP do not regulate each other's expression in mice. (A) The expression level of MeCP2 was not altered in *Ube3a*-null mice. (B) The expression level of E6AP was not altered in *MECP2*-TG mice while MeCP2 expression was increased by >2-fold. (C) E6AP expression level was not changed in *Mecp2*-null male mice. Graphs show the normalized levels (wild-type = 100%) of MeCP2 (A) and E6AP (B, C) in whole brain extracts from wild-type or mutant mice. WT = wild-type. Tg = Transgenic. NS = non-significant, *t*-test.

absence of E6AP. We first analyzed the protein level of Sst in wild-type and *Ube3a*-null hypothalami, a brain region with several well-validated mRNA transcripts that are up-regulated by MeCP2 overexpression [10]. We found that Sst protein levels were significantly decreased in *Ube3a*-null hypothalami compared with those of their wild-type littermates (Fig. 3B and C). Importantly, we also observed that increased Sst expression driven by *MECP2* overexpression in *MECP2*-TG mice was strongly suppressed by loss of the maternal *Ube3a* allele (Fig. 3E). Consistent with E6AP's role as a transcriptional coactivator, the mRNA expression levels of Sst were significantly decreased in the *Ube3a*-null hypothalami compared to those of their wild-type littermates (Fig. 3D). It was also reported that *Sst* transcription was down-regulated in the $Ube3a^{m-/p+}$ cerebellum compared to wild-type mice [26]. Taken together, these data suggest that E6AP can function as a coactivator for the MeCP2-mediated regulation of Sst expression *in vivo*.

In contrast to Sst, the protein and mRNA expression levels of CREB1, another validated MeCP2-activated target [10], were not altered in *Ube3a*-null hypothalami (Fig. 3B–D). This suggests that E6AP may not cooperate with MeCP2 in CREB1 expression and raises the possibility that expression of a subset, but not all, of MeCP2 targets depends on E6AP activity. To determine what percent of activated MeCP2 targets are affected by E6AP function, we randomly selected 60 genes whose levels were increased in MeCP2-TG mice [10]. By quantitative real-time PCR analysis, we found that 6 out of 62 genes tested were down-regulated while 13 genes were up-regulated in the *Ube3a*-null hypothalami (Supplementary Table S1), suggesting that about 10 percent of the genes that we analyzed are normally activated by MeCP2 and E6AP in this brain region.

3.4. E6AP does not regulate the expression of known corepressors for MeCP2

We next investigated the mechanism by which E6AP enhances MeCP2-mediated regulation of gene expression. Since E6AP acts as an E3 ubiquitin ligase, it is possible that E6AP may promote the degradation of some negative regulators of MeCP2, allowing MeCP2 to be available to its coactivators and to activate target genes. MeCP2 has been known to associate with corepressor complexes containing mSin3A, histone deacetylases, and CoREST [12,13]. To test whether E6AP targets these corepressors for degradation, we checked the protein levels of mSin3A and CoREST in whole brain, as well as specific brain regions including hypothalamus, olfactory bulb, amygdala, and others, of *Ube3a*-null mice compared with their wild-type littermates, but we found no differences between the genotypes (Supplementary Fig. S1). This observation rules out the possibility that the degradation of at least these corepressors could be linked to E6AP-mediated transcriptional activation of MeCP2 targets.

3.5. E6AP physically associates with MeCP2

Another mechanism by which E6AP could activate MeCP2 targets is to act as a coactivator directly by associating with MeCP2. In support of this, we found that MeCP2 physically interacted with all three isoforms of E6AP in cultured mammalian cells by co-immunoprecipitation (co-IP) assays (Fig. 4A). We also performed co-IP assays on mouse brain extracts using a specific E6AP antibody. To increase IP efficiency of MeCP2-associated protein complexes *in vivo*, we generated a P1 artificial chromosome (PAC) transgenic mouse that mildly overexpresses *MECP2-GFP* under the control of its endogenous promoter by one and a half fold the wild-type level (Chahrour and Zoghbi, unpublished data). The anti-E6AP antibody co-immunoprecipitated MeCP2-GFP from whole brain extracts (Fig. 4B), suggesting that MeCP2 and E6AP do indeed interact *in vivo*. These data support the idea that E6AP directly associates with MeCP2.

4. Discussion

RTT and AS display many overlapping neurological phenotypes. Extensive studies have been performed to determine the cause of neurological problems. However, the genetic and molecular basis of the overlap in phenotypes remains largely unknown. In this study we provide *in vivo* genetic and molecular evidence that MeCP2 and E6AP share functions by demonstrating that they are involved in the regulation of shared target gene expression. First, our genetic interaction studies show that MeCP2 overexpression phenotypes are improved by decreased expression of *Ube3a* in *Drosophila* (Fig. 1). These data suggest that MeCP2 activity is dependent on E6AP and further suggest that MeCP2 and E6AP may affect some overlapping genetic pathways. Second, we show that MeCP2 and E6AP do not regulate each other's expression (Fig. 2). Furthermore, E6AP does not affect expression levels of some of MeCP2-associated proteins in mice *in vivo* (Supplementary Fig. S1), which are crucial for the regulation of MeCP2-mediated target gene expression. There is still the possibility of novel

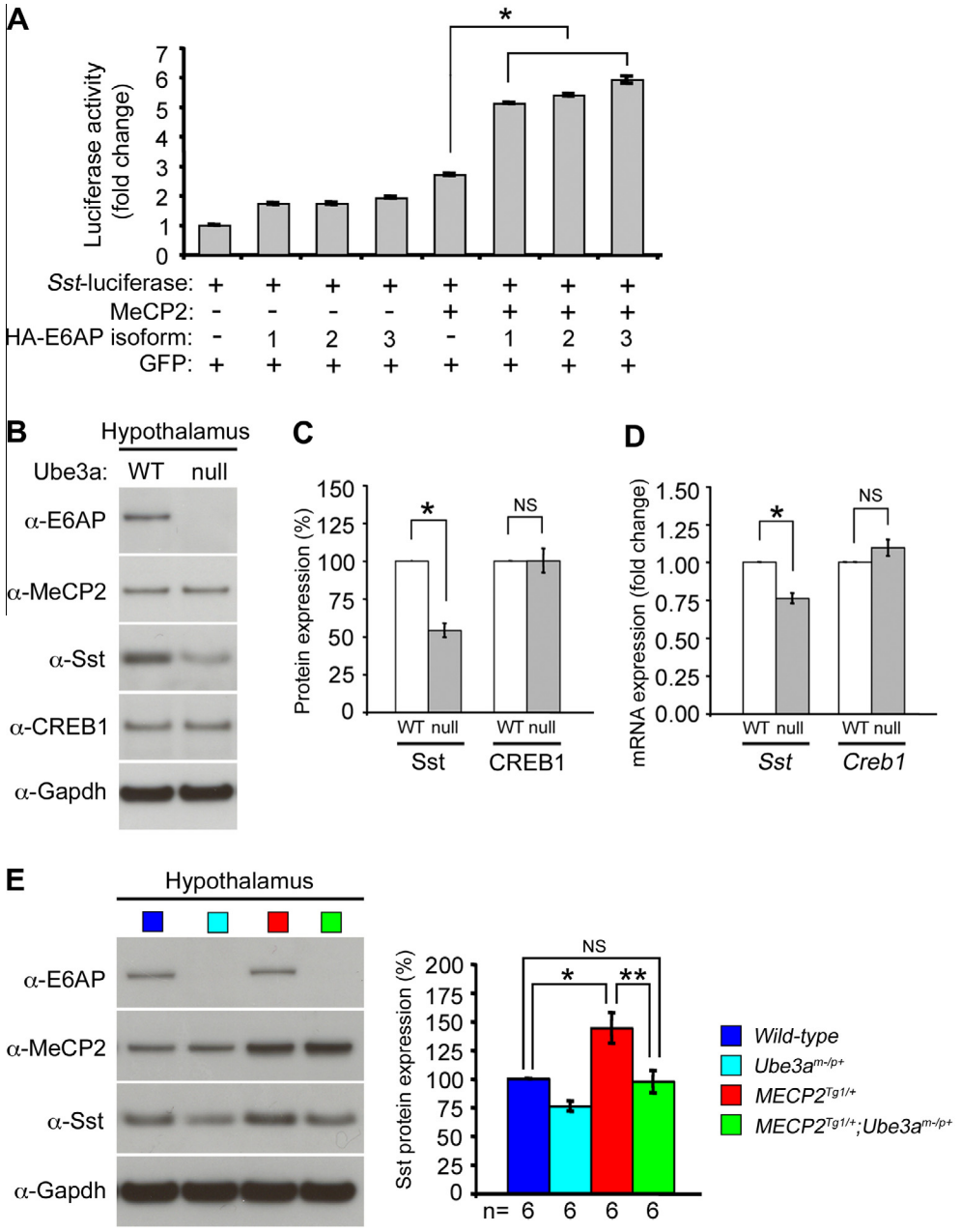

Fig. 3. E6AP functions as a coactivator for MeCP2-mediated gene expression. (A) Luciferase assay shows that MeCP2 and E6AP could synergistically activate Sst promoter in HEK293T cells ($n = 3$, $^{*}P < 7.53 \times 10^{-8}$, two-way ANOVA). (B) The expression level of Sst, but not CREB1, was significantly decreased in the hypothalamus of a representative *Ube3a*-null mouse compared to its wild-type (WT) littermate. (C and D) Graphs show the normalized expression levels of Sst and CREB1 proteins ($n = 5$, $^{*}P < 0.001$, *t*-test) or mRNAs ($n = 3$, $^{*}P < 0.005$, *t*-test) in hypothalami from wild-type or *Ube3a*-null mice. NS = non-significant, *t*-test. (E) *MECP2* overexpression-induced Sst up-regulation was strongly suppressed by loss of maternal *Ube3a* expression. Graph shows the normalized expression levels of Sst protein in hypothalami ($^{*}P < 0.01$, $^{**}P < 0.005$, NS = non-significant, one-way ANOVA). Genotypes are color-coded. (For interpretation of color in this Figure, the reader is referred to the web version of this article).

unidentified MeCP2 corepressors regulated by E6AP, however. Rather, MeCP2 and E6AP can physically associate with each other and modulate expression of some common target genes (Figs. 3 and 4). MeCP2 and E6AP may co-regulate gene expression by acting directly at the promoter and/or regulatory elements of their target genes. Alternatively, E6AP may affect MeCP2 function in organization of chromatin structures [14–16] and gene expression changes might be secondary to the altered chromatin structures. Regardless, MeCP2 and E6AP appear to co-regulate the expression of about 10% of MeCP2 target genes in the hypothalamus that we tested (Supplementary Table S1). Although the gene expression changes may not directly explain specific behavioral and neurological phenotypes at this moment, these molecular data are in agreement with our genetic interaction data in that loss of (or decrease in) *Ube3a* expression suppresses *MECP2* overexpression-induced phenotypes. This co-regulation of shared molecular targets between MeCP2 and E6AP may explain the similarities in disease features of RTT and AS.

Our findings, however, should not diminish the importance of other functions of MeCP2 or E6AP and the contributions of such functions to RTT and AS or even ASDs. Only some MeCP2-activated targets were influenced by E6AP coactivator activity, suggesting that MeCP2 may be able to form several different transcriptional complexes with different proteins besides E6AP to regulate

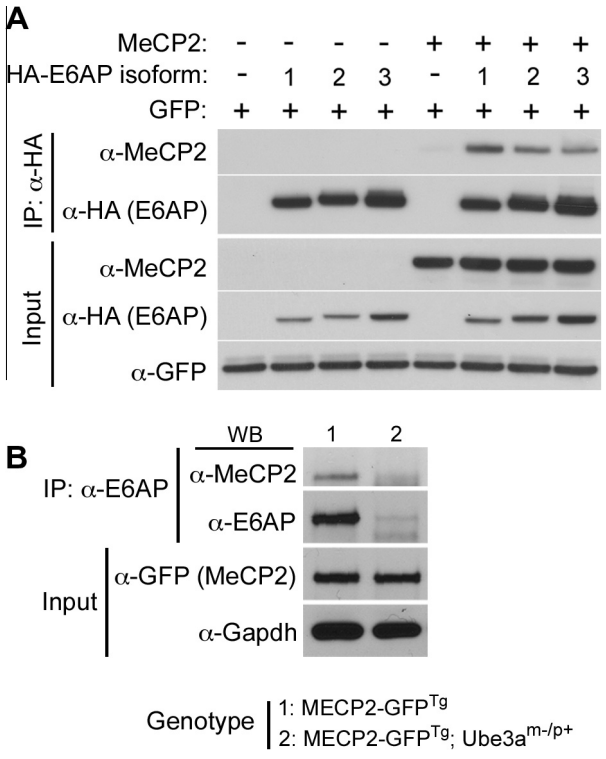

Fig. 4. MeCP2 associates with E6AP. (A) MeCP2 interacted with all three isoforms of E6AP in HEK293T cells by co-immunoprecipitation (co-IP) assays. Top panel shows expression of MeCP2 after IP using anti-HA-agarose beads, demonstrating the MeCP2-E6AP interaction. GFP expression was used as a transfection and loading control. (B) Interaction of MeCP2 with E6AP in mouse brain. The anti-E6AP antibody was used to co-immnoprecipitate MeCP2.

expression of distinct groups of target genes. In addition, the transcriptional repressor function of MeCP2 may also play a crucial role in the pathogenesis of RTT. This may also be the case for E6AP since it may function as a coactivator for several different transcription factors [17] besides MeCP2. Furthermore, the E3-ubiquitin ligase function is also crucial for E6AP function, as well as the pathogenesis of AS [28–30]. Altogether, a detailed understanding of the molecular functions and the crosstalk of MeCP2 and E6AP with other autism-causing proteins will be at the center of future studies aimed at understanding these protein functions in brain, as well as for developing interventions for RTT, AS, and ASDs.

Acknowledgments

This work was supported by the Charles H. Hood Foundation; Yale Scholar Award program; NARSAD Young Investigator Award; and the Alfred P. Sloan Foundation. We would like to thank Drs. Zoghbi and Beaudet for providing access to *MECP2* and *Ube3a* transgenic and mutant mice. We are also grateful to Dr. Fischer for *dUbe3a* mutant flies and Dr. Botas for *MECP2* transgenic fly, Dr. Mandel for the anti-CoREST antibody. We thank Dr. Zoghbi and members of the Zoghbi and Lim laboratory for critical reading of this manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.06.036.

References

[1] R.E. Amir, I.B. Van den Veyver, M. Wan, C.Q. Tran, U. Francke, H.Y. Zoghbi, Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2, Nat. Genet. 23 (1999) 185–188.
[2] J. Clayton-Smith, L. Laan, Angelman syndrome: a review of the clinical and genetic aspects, J. Med. Genet. 40 (2003) 87–95.
[3] T. Kishino, M. Lalande, J. Wagstaff, UBE3A/E6-AP mutations cause Angelman syndrome, Nat. Genet. 15 (1997) 70–73.
[4] T. Matsuura, J.S. Sutcliffe, P. Fang, R.J. Galjaard, Y.H. Jiang, C.S. Benton, J.M. Rommens, A.L. Beaudet, De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome, Nat. Genet. 15 (1997) 74–77.
[5] J.M. Huibregtse, M. Scheffner, S. Beaudenon, P.M. Howley, A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase, Proc. Natl. Acad. Sci. USA 92 (1995) 2563–2567.
[6] F. Ariani, F. Mari, C. Pescucci, I. Longo, M. Bruttini, I. Meloni, G. Hayek, R. Rocchi, M. Zappella, A. Renieri, Real-time quantitative PCR as a routine method for screening large rearrangements in Rett syndrome: report of one case of MECP2 deletion and one case of MECP2 duplication, Hum. Mutat. 24 (2004) 172–177.
[7] E.H. Cook Jr., V. Lindgren, B.L. Leventhal, R. Courchesne, A. Lincoln, C. Shulman, C. Lord, E. Courchesne, Autism or atypical autism in maternally but not paternally derived proximal 15q duplication, Am. J. Hum. Genet. 60 (1997) 928–934.
[8] X. Nan, F.J. Campoy, A. Bird, MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin, Cell 88 (1997) 471–481.
[9] J.I. Young, E.P. Hong, J.C. Castle, J. Crespo-Barreto, A.B. Bowman, M.F. Rose, D. Kang, R. Richman, J.M. Johnson, S. Berget, H.Y. Zoghbi, Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2, Proc. Natl. Acad. Sci. USA 102 (2005) 17551–17558.
[10] M. Chahrour, S.Y. Jung, C. Shaw, X. Zhou, S.T. Wong, J. Qin, H.Y. Zoghbi, MeCP2, a key contributor to neurological disease, activates and represses transcription, Science 320 (2008) 1224–1229.
[11] S.W. Long, J.Y. Ooi, P.M. Yau, P.L. Jones, A brain-derived MeCP2 complex supports a role for MeCP2 in RNA processing, Biosci. Rep. 31 (2011) 333–343.
[12] K.N. Harikrishnan, M.Z. Chow, E.K. Baker, S. Pal, S. Bassal, D. Brasacchio, L. Wang, J.M. Craig, P.L. Jones, S. Sif, A. El-Osta, Brahma links the SWI/SNF chromatin-remodeling complex with MeCP2-dependent transcriptional silencing, Nat. Genet. 37 (2005) 254–264.
[13] N. Ballas, C. Grunseich, D.D. Lu, J.C. Speh, G. Mandel, REST and its corepressors mediate plasticity of neuronal gene chromatin throughout neurogenesis, Cell 121 (2005) 645–657.
[14] S.A. Baker, L. Chen, A.D. Wilkins, P. Yu, O. Lichtarge, H.Y. Zoghbi, An AT-hook domain in MeCP2 determines the clinical course of Rett syndrome and related disorders, Cell 152 (2013) 984–996.
[15] M. Mellen, P. Ayata, S. Dewell, S. Kriaucionis, N. Heintz, MeCP2 binds to 5hmC enriched within active genes and accessible chromatin in the nervous system, Cell 151 (2012) 1417–1430.
[16] P.J. Skene, R.S. Illingworth, S. Webb, A.R. Kerr, K.D. James, D.J. Turner, R. Andrews, A.P. Bird, Neuronal MeCP2 is expressed at near histone-octamer levels and globally alters the chromatin state, Mol. Cell 37 (2010) 457–468.
[17] Z. Nawaz, D.M. Lonard, C.L. Smith, E. Lev-Lehman, S.Y. Tsai, M.J. Tsai, B.W. O'Malley, The Angelman syndrome-associated protein, E6-AP, is a coactivator for the nuclear hormone receptor superfamily, Mol. Cell. Biol. 19 (1999) 1182–1189.
[18] J. Lim, T. Hao, C. Shaw, A.J. Patel, G. Szabo, J.F. Rual, C.J. Fisk, N. Li, A. Smolyar, D.E. Hill, A.L. Barabasi, M. Vidal, H.Y. Zoghbi, A protein–protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration, Cell 125 (2006) 801–814.
[19] S. Warming, N. Costantino, D.L. Court, N.A. Jenkins, N.G. Copeland, Simple and highly efficient BAC recombineering using galK selection, Nucleic Acids Res. 33 (2005) e36.
[20] A.L. Collins, J.M. Levenson, A.P. Vilaythong, R. Richman, D.L. Armstrong, J.L. Noebels, J. David Sweatt, H.Y. Zoghbi, Mild overexpression of MeCP2 causes a progressive neurological disorder in mice, Hum. Mol. Genet. 13 (2004) 2679–2689.
[21] Y.H. Jiang, D. Armstrong, U. Albrecht, C.M. Atkins, J.L. Noebels, G. Eichele, J.D. Sweatt, A.L. Beaudet, Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation, Neuron 21 (1998) 799–811.
[22] H. Ju, H. Kokubu, T.W. Todd, J.J. Kahle, S. Kim, R. Richman, K. Chirala, H.T. Orr, H.Y. Zoghbi, J. Lim, Polyglutamine disease toxicity is regulated by nemo-like kinase in spinocerebellar ataxia type 1, J. Neurosci. 33 (2013) 9328–9336.
[23] W.H. Kao, S.L. Beaudenon, A.L. Talis, J.M. Huibregtse, P.M. Howley, Human papillomavirus type 16 E6 induces self-ubiquitination of the E6AP ubiquitin-protein ligase, J. Virol. 74 (2000) 6408–6417.
[24] Y. Wu, F.V. Bolduc, K. Bell, T. Tully, Y. Fang, A. Sehgal, J.A. Fischer, A Drosophila model for Angelman syndrome, Proc. Natl. Acad. Sci. USA 105 (2008) 12399–12404.
[25] H.N. Cukier, A.M. Perez, A.L. Collins, Z. Zhou, H.Y. Zoghbi, J. Botas, Genetic modifiers of MeCP2 function in Drosophila, PLoS Genet. 4 (2008) e1000179.
[26] D. Low, K.S. Chen, Genome-wide gene expression profiling of the Angelman syndrome mice with Ube3a mutation, Eur. J. Hum. Genet. 18 (2010) 1228–1235.

[27] S. Kuhnle, B. Mothes, K. Matentzoglu, M. Scheffner, Role of the ubiquitin ligase E6AP/UBE3A in controlling levels of the synaptic protein Arc, Proc. Natl. Acad. Sci. USA 110 (2013) 8888–8893.
[28] D. Zaaroor-Regev, P. de Bie, M. Scheffner, T. Noy, R. Shemer, M. Heled, I. Stein, E. Pikarsky, A. Ciechanover, Regulation of the polycomb protein Ring1B by self-ubiquitination or by E6-AP may have implications to the pathogenesis of Angelman syndrome, Proc. Natl. Acad. Sci. USA 107 (2010) 6788–6793.
[29] S.S. Margolis, J. Salogiannis, D.M. Lipton, C. Mandel-Brehm, Z.P. Wills, A.R. Mardinly, L. Hu, P.L. Greer, J.B. Bikoff, H.Y. Ho, M.J. Soskis, M. Sahin, M.E. Greenberg, EphB-mediated degradation of the RhoA GEF Ephexin5 relieves a developmental brake on excitatory synapse formation, Cell 143 (2010) 442–455.
[30] P.L. Greer, R. Hanayama, B.L. Bloodgood, A.R. Mardinly, D.M. Lipton, S.W. Flavell, T.K. Kim, E.C. Griffith, Z. Waldon, R. Maehr, H.L. Ploegh, S. Chowdhury, P.F. Worley, J. Steen, M.E. Greenberg, The Angelman Syndrome protein Ube3A regulates synapse development by ubiquitinating arc, Cell 140 (2010) 704–716.