Mi-2β Associates with BRG1 and RET Finger Protein at the Distinct Regions with Transcriptional Activating and Repressing Abilities*

Yohei Shimono‡, Hideki Murakami‡, Kumi Kawai§, Paul A. Wade¶, Kaoru Shimokata‡, and Masahide Takahashi¶**

From the Departments of ‡Pathology and Internal Medicine, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan, §Department of Molecular Pathology, Center for Neural Disease and Cancer, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan, and ¶Department of Pathology, Emory University, Atlanta, Georgia 30322

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Mi-2β is the main component of the nucleosome remodeling and deacetylase complex and plays an important role in epigenetic transcriptional repression. Here we show that the amino-terminal and carboxyl-terminal regions of Mi-2β have distinct transcriptional activities and bind to BRG1, a component of the SWI/SNF complex, and the RET finger protein (RFP), respectively. Analysis by luciferase reporter assay revealed that the amino-terminal region of Mi-2β has a strong transactivating ability, whereas its carboxyl-terminal region has transcriptional repressive activity. Co-localization and association of Mi-2, RFP, and histone deacetylase 1 suggested that these proteins cooperate in transcriptional repression. Furthermore, the functional importance of the association of Mi-2β and RFP was confirmed by using RFP−/− fibroblasts. On the other hand, we demonstrated that Mi-2 and BRG1 were associated with each other and that the bromodomain region of BRG1 strongly suppressed transactivation by the amino-terminal region of Mi-2β. The findings that Mi-2β interacts with both transactivating and repressing proteins and directly associates with another chromatin remodeling protein, BRG1, provide new insight into the formation of multiprotein supercomplex involved in transcriptional regulation.

Epigenetic control of gene expression contributes to the genome-wide mechanism of information storage and retrieval by changing chromatin structure (1). Emerging evidence suggests that dynamic changes in chromatin structure, including heterochromatin and euchromatin formation, modification of histone tails, ATP-dependent nucleosome remodeling activity, and binding of Polycomb and trithorax group proteins, play an important role in transcriptional regulation by changing the access to the underlying DNA (2–4).

Mi-2 is the main component of the nucleosome remodeling and deacetylase (NuRD)1 complex and is an autoantigen of dermatomyositis that is strongly associated with cancer (5, 6).

Mi-2β is involved in transcriptional repression, in cooperation with its associating proteins such as methyl CpG-binding protein MBD2/3, histone deacetylases HDAC1/2, MTA1/2/3, and RbAp46/48 (7–10). The NuRD complex associates with distinct types of transcriptional repressors. In particular, the carboxyl-terminal region of Mi-2 was identified as a binding site for several transcriptional repressors including hunchback, Trk69 (tram-track 69), and KAP-1 co-repressor (11–13). In addition, recent work has shown that Mi-2 is a component of the cohesin complex that functions in sister chromatin segregation (14).

The RET finger protein (RFP) was originally identified as a protein that became oncogenic after fusing with the RET receptor tyrosine kinase (15). RFP belongs to the RBCC-B30.2 protein family that contains a RING finger, a B-box zinc finger, a coiled-coil domain, and a specific carboxyl-terminal region known as the RFP domain or the B30.2-like domain (16). We reported previously (15, 17) that RFP mRNA was highly expressed in a variety of human and rodent tumor cell lines, as well as in male germ cells, and that its protein expression was detected in the nuclei of male germ cells, peripheral and central neurons, hepatocytes, and adrenal chromaffin cells. Moreover, our recent study provided evidence that RFP binds and co-localizes with EPC1 (Enhancer of Polycomb 1) in the nucleus and is involved in transcriptional repression (18, 19). The repressive activity of RFP resided mainly in its coiled-coil domain, which represents the binding site for EPC1.

BRG1 is a central subunit of the SWI/SNF complex that participates in transcriptional activation (20, 21). The SWI/SNF complex is recruited to promoters of specific genes, where it remodels nucleosomes to facilitate the binding of transcriptional activators. Interestingly, recent results show that the SWI/SNF complex also has transcriptional repressing activity in Drosophila and yeast (22, 23) and that BRG1 interaction with HP1 (heterochromatin-associated protein 1) enhances transcriptional repression in mammalian cells (24). In addition, BRG1 modulates the transcriptional activity of retinoblastoma, β-catenin, the estrogen receptor, and BRCA1 and is mutated or deleted in a variety of human tumor cell lines (23, 25–28).

Whereas Mi-2 and BRG1 are the main subunits of the well known protein complexes NuRD and SWI/SNF, co-existence of SWI/SNF and NuRD components has been reported in several multiprotein complexes. An Ikaros-containing chromatin remodeling complex that is involved in γ- to β-globin switching in hematopoietic cells includes Mi-2, BRG1, HDAC, RbAp46/48, and BAF57 in a single complex (29). ALL-1, a trithorax/Polycomb

DBD, DNA-binding domain; 3-AT, 3-amino-1,2,4-triazole; SRE, serum response element; CRE, cyclic AMP response element.
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comb group protein that is involved in acute leukemia, was reported recently (30) to form the multiprotein supercomplex that includes both Mi-2 and Brahma that is highly homologous to BRG1.

Here we provide evidence that Mi-2β has both transcriptional repressing and activating domains. RFP directly binds the carboxyl-terminal region of Mi-2β, and the transcriptional repressing activity of RFP is enhanced by Mi-2β in a dependent manner. In addition, the transcriptional repressing activity of Mi-2β was markedly weakened in Rfp/−/ fibroblasts. On the other hand, the amino-terminal region of Mi-2β has a strong transcriptional activating ability and contains a binding site for BRG1. These data indicate that Mi-2 can cooperate with both transactivating and repressing proteins at distinct regions. These findings suggest that the direct association of chromatin remodeling proteins such as Mi-2 and BRG1 could mediate formation of multiprotein supercomplexes involved in epigenetic transcription.

EXPERIMENTAL PROCEDURES

Plasmids—Fragments of RFP cDNA containing the RING finger B box region, the coiled-coil region, or the RFP domain region were cloned into the vectors as described previously (18). Mi-2 β cDNA cloned into the pBS or pcDNA3 HA vector was a kind gift from F. Hirose (Aichi Cancer Center, Nagoya, Japan). Each region of Mi-2β was amplified by PCR using 5′ primers with an EcoRI and BamHI site and 3′ primers with a NotI site. The resulting PCR products were subcloned into the pGEM-T vector (Promega) and sequenced. Each product was inserted into the EcoRI/NotI or BamHI/NotI site of the pcDNA3.1V5 HisC GAL4 DBD vector (18) and then subcloned into the EcoRI/Xhol or BamHI/ Xhol site of the pACT2 vector. Mi-2β cDNA was also cloned into the EcoRIV/SAI site of the pFLAG CMV2 vector.

The carboxyl-terminal region of the BRG1 cDNA was obtained by PCR using the human testis cDNA library as a template and was sequenced. BRG1 cDNA fragments were amplified by PCR using 5′ primers with an EcoRI site and 3′ primers with a Xhol site and were cloned into the pGEM-T vector. The resulting cDNA fragments were subcloned into the EcoRI/Xhol site of the pACT2 vector. To generate pFLAG CMV2 BRG1 expression plasmids, each BRG1 cDNA fragment was subcloned into the EcoRIV/SAI site of the pFLAG CMV2 vector.

Yeast Two-hybrid Screening and Interaction Assays—Full-length RFP cDNA or the amino-terminal region of Mi-2β cDNA was cloned into the pAS2–1 vector (Clontech) and used as a bait to identify interacting proteins by a yeast two-hybrid assay. The pAS2–1 RFP or Mi-2β plasmid was co-transfected with a human testis cDNA library (Clontech) into the Y190 strain of Saccharomyces cerevisiae. The transformants were plated on selective medium lacking histidine, tryptophan, and leucine with 40 mM 3-amino-1,2,4-triazole (3-AT). From −1.4 × 105 independent colonies, 15 positive colonies were isolated by their growth on minimal medium lacking leucine, tryptophan, and histidine and by β-galactosidase expression. β-Galactosidase activity was measured by the filter assay method according to the manufacturer’s instructions (Clontech). After DNA isolation, these clones were further characterized by sequencing and analyzed for gene homology using the BLAST data base.

To characterize the interaction between RFP and Mi-2β in the yeast two-hybrid system, each RFP and Mi-2β domain amplified by PCR was cloned into pAS2–1 and pACT2, respectively, and transformed into Y190. Positive interactions were also determined by two criteria of the yeast two-hybrid system, each region was co-transfected with a human testis cDNA library (Clontech). Mi-2 is the main component of the NuRD complex, which is known to be involved in gene silencing. Mi-2 has two isoforms, Mi-2α and Mi-2β, that possesses plant homeodomain zinc finger motifs, a chromodomain, and an ATPase/helicase region (Fig. 1A). The isoforms show a 69% amino acid identity. To clarify the interacting domains between RFP and Mi-2β, different fragments of RFP and Mi-2β cDNAs were cloned in-frame into pAS2–1 and pACT2 vectors, respectively. Because library screening showed that the full-length RFP interacts with the carboxyl-terminal region of Mi-2β, we chose the carboxyl-terminal half of Mi-2β for further analysis. pAS2–1 RFP and pACT2 Mi-2β constructs were transformed into the yeast Y190 strain, and interactions were assayed both by growth on selective medium lacking histidine, tryptophan, and leucine with 40 mM 3-AT and by β-galactosidase activity. As shown in Fig. 1B, a strong interaction was detected between the RFP coiled-coil domain and the Mi-2β carboxyl-terminal fragments (fragments D and H). On the other hand, the constructs without the carboxyl-terminal region (A, B, C, and E in Fig. 1A) had very weak or no binding activity, confirming that the D region is important and specific for RFP binding. The carboxyl-terminal region of Mi-2 has been reported to be the binding site for several repressors such as hunchback, Trk69, and Kap-1 (11–13), and our results support the idea that this

RESULTS

Identification of Interacting Domains between RFP and Mi-2β—We have shown previously (18) that RFP is involved in epigenetic gene silencing, cooperating, and co-localizing with EPC1. To further identify RFP-binding proteins, we performed yeast two-hybrid screening using full-length RFP cDNA as bait. Carboxyl-terminal fragments of Mi-2β cDNA were isolated from a human testis Matchmaker two-hybrid library (Clontech). Mi-2 is the main component of the NuRD complex, which is known to be involved in gene silencing. Mi-2 has two isoforms, Mi-2α and Mi-2β, that possesses plant homeodomain zinc finger motifs, a chromodomain, and an ATPase/helicase region (Fig. 1A). The isoforms show a 69% amino acid identity. To clarify the interacting domains between RFP and Mi-2β, different fragments of RFP and Mi-2β cDNAs were cloned in-frame into pAS2–1 and pACT2 vectors, respectively. Because library screening showed that the full-length RFP interacts with the carboxyl-terminal region of Mi-2β, we chose the carboxyl-terminal half of Mi-2β for further analysis. pAS2–1 RFP and pACT2 Mi-2β constructs were transformed into the yeast Y190 strain, and interactions were assayed both by growth on selective medium lacking histidine, tryptophan, and leucine with 40 mM 3-AT and by β-galactosidase activity. As shown in Fig. 1B, a strong interaction was detected between the RFP coiled-coil domain and the Mi-2β carboxyl-terminal fragments (fragments D and H). On the other hand, the constructs without the carboxyl-terminal region (A, B, C, and E in Fig. 1A) had very weak or no binding activity, confirming that the D region is important and specific for RFP binding. The carboxyl-terminal region of Mi-2 has been reported to be the binding site for several repressors such as hunchback, Trk69, and Kap-1 (11–13), and our results support the idea that this
ON SELECTIVE MEDIUM WITH 40 mM 3-AT AND BY REDUCED INTO YEAST Y190 CELLS. ASSOCIATIONS WERE ASSESSED BOTH BY GROWTH TERTIVELY, AND USED FOR YEAST TWO-HYBRID ASSAYS. DOMAIN STRUCTURES OR FOURS INDEPENDENT COLONIES.

THE DEGREE OF ASSOCIATION IS GRADED AS STRONG (+), MODERATE (+/−), WEAK (+/−), OR NEGATIVE (−). MEASUREMENTS WERE PERFORMED USING AT LEAST FOUR INDEPENDENT COLONIES.

REGION PROVIDES COMMON BINDING SITES FOR DIFFERENT TYPES OF REPRESSORS, INCLUDING RFP.

ASSOCIATION AND CO-LOCALIZATION OF RFP, MI-2, AND HDAC1—TO VERIFY THE INTERACTION BETWEEN RFP AND MI-2, IMMUNOPRECIPITATION EXPERIMENTS WERE PERFORMED USING HUMAN EMBRYO KIDNEY 293 CELLS, IN WHICH THE EXPRESSION OF BOTH PROTEINS WAS DETECTED BY WESTERN BLOTTING (18). WE ALSO EXAMINED THE ASSOCIATION OF RFP WITH HDAC1 THAT IS KNOWN TO BE A COMPONENT OF THE NuRD COMPLEX. WHEN THE CELL LYSATES WERE IMMUNOPRECIPITATED WITH AN ANTI-RFP ANTIBODY, BOTH MI-2 AND HDAC1 WERE DETECTED IN THE IMMUNOPRECIPITATES (Fig. 2), SUGGESTING THE ASSOCIATION OF THESE THREE PROTEINS. THESE INTERACTIONS WERE NOT OBSERVED WHEN NORMAL RABBIT IMMUNOGLOBULINS WERE USED FOR IMMUNOPRECIPITATION (Fig. 2).

WE NEXT ANALYZED THE SUBCELLULAR LOCALIZATION OF RFP, MI-2, AND HDAC1 BY IMMUNOFLUORESCENCE. TO DETERMINE THE ENDOGENOUS LOCALIZATION OF THESE PROTEINS, SW480 HUMAN COLORECTAL ADENOCARCINOMA CELLS, IN WHICH THE EXPRESSION OF THESE PROTEINS WAS DETECTED BY WESTERN BLOTTING (DATA NOT SHOWN), WERE STAINED WITH AN ANTI-MI-2 POLYCLONAL ANTIBODY, AN Alexa 546-conjugated Anti-RFP POLYCLONAL ANTIBODY, AND AN ANTI-HDAC1 MONOCLONAL ANTIBODY. THE LOCALIZATION PATTERNS OF THESE THREE PROTEINS WERE CHARACTERIZED BY DIFFUSE DISTRIBUTION THROUGHOUT THE NUCLEOLUS, DOT-LIKE STRUCTURES OF VARIOUS SIZES, AND BRIGHTLY LABELED DOMAIN FORMATION (Fig. 3, A–C). SUPERIMPOSITION OF CONFOCAL IMAGES REVEALED THAT RFP, MI-2, AND HDAC LARGELY CO-LOCALIZED IN THESE NUCLEAR STRUCTURES.

ANALYSIS OF TRANSCRIPTIONAL ACTIVITY OF MI-2β—BECAUSE THE CARBOXYL-TERMINAL REGION OF MI-2 IS THE BINDING SITE FOR VARIOUS TRANSCRIPTIONAL REPRESSORS INCLUDING RFP, A PANEL OF MI-2β FRAGMENTS FUSED TO A GAL4 DBD WAS GENERATED TO EVALUATE THE TRANSCRIPTIONAL ACTIVITY OF THIS REGION IN COMPARISON WITH OTHER REGIONS OF MI-2β (Fig. 4A). FOUR DIFFERENT LUCFERENCE REPORTER CONSTRUCTS WERE USED, EACH CONTAINING FIVE TANDEM REPEATS OF THE GAL4 BINDING SEQUENCE, BINDING SITES FOR TRANSCRIPTIONAL ENHANCERS (SERUM RESPONSE ELEMENT (SRE) OR CYCLIC AMP RESPONSE ELEMENT (CRE)), AND A SV40 PROMOTER OR HERPES SIMPLEX VIRUS-THYMIDINE KINASE MINIMAL PROMOTER (Fig. 4A). THE TRANSCRIPTIONAL ACTIVITIES WERE ASSESSED BY TRANSIENT TRANSFECTION OF EACH GAL4 DBD-MI-2β CONSTRUCT, TOGETHER WITH FOUR DIFFERENT LUCFERENCE REPORTER PLASMIDS, INTO 293 CELLS. IN THE EXPERIMENTS USING THE CRE REPORTER CONSTRUCT, THE CELLS WERE TREATED WITH 100 μM FORSKOLIN FOR 2 H.

AS WE HAVE SHOWN PREVIOUSLY (18), RFP REDUCED THE LUCFERENCE ACTIVITY BY −65–75% AS COMPARED WITH THE CONTROL (GAL4 DBD ONLY) (Fig. 4B). FULL-LENGTH MI-2β ALSO REDUCED THE ACTIVITY BY 55–70%. WE FOUND THAT THE CARBOXYL-TERMINAL FRAGMENT (FRAGMENT J; 1321–1912 AMINO ACIDS) THAT INCLUDES THE RFP BINDING REGION HAS A TRANSCRIPTIONAL REPRESSIVE ACTIVITY SIMILAR TO THAT OF RFP, INDEPENDENT OF THE DIFFERENCES OF THE REPORTER PLASMIDS USED (Fig. 4B). THE CHROMODOMAIN REGION (FRAGMENT E) ALSO SHOWED REPRESSIVE ACTIVITY (ACTIVITY REDUCED BY 40–70%), WHEREAS OTHER REGIONS (FRAGMENTS D, F, AND G), INCLUDING THE PLANT HOMEODOMAIN ZINC FINGER REGION AND THE ATPASE/Helicase Region, HAD WEAK OR NO REPRESSIVE ACTIVITY. TO FURTHER CHARACTERIZE THE REPRESSIVE ACTIVITY OF THE CARBOXYL-TERMINAL REGION, WE MADE ADDITIONAL DELETION CONSTRUCTS OF THIS REGION. AS SHOWN IN Fig. 4C, THE REGION BETWEEN 1671 AND 1912 AMINO ACIDS (FRAGMENT N) WAS NECESSARY FOR MAXIMAL REPRESSIVE ACTIVITY.

FIG. 2. INTERACTION OF ENDOGENOUS RFP WITH MI-2 AND HDAC IN VIVO. THE LYSATE FROM HUMAN EMBRYONIC KIDNEY 293 CELLS WAS IMMUNOPRECIPITATED (IP) WITH AN ANTI-RFP ANTIBODY OR WITH NORMAL RABBIT IMMUNOGLOBULINS (CONTROL), FOLLOWED BY IMMUNOBLOTTING WITH AN ANTI-MI-2 OR ANTI-HDAC1 ANTIBODY. THE WHOLE CELL LYSATE WAS USED FOR THE IMMUNOPRECIPITATION.

FIG. 3. A–C. SUPERIMPOSITION OF CONFOCAL IMAGES REVEALED THAT RFP, MI-2, AND HDAC LARGELY CO-LOCALIZED IN THESE NUCLEAR STRUCTURES.
Cooperation of RFP and Mi-2β in Transcriptional Repression—We have succeeded recently in generating Rfp knockout mice. As shown in Fig. 5A, the transcriptional repressive activity of the full-length Mi-2β was almost abolished in Rfp−/− fibroblasts. Its carboxyl-terminal region (fragment J; 1321–1912 amino acids), which has a repressing activity in 293 cells (Fig. 4B), instead showed transcriptional activating ability in Rfp−/− fibroblasts. In contrast, the activity of fragment F of Mi-2β was not affected in Rfp−/− fibroblasts (Fig. 5A). It is noteworthy that co-expression of RFP markedly restored the repressive activities of both full-length Mi-2β and its carboxyl-terminal fragment J in Rfp−/− fibroblasts (Fig. 5A).

In addition, co-expression of full-length Mi-2β enhanced the transcriptional repressive activity of RFP in 293 cells (Fig. 5B). These results strongly argue that RFP plays a role in Mi-2β-mediated transcriptional repression.

**Strong Transactivating Ability of the Amino Terminal Region of Mi-2β and Its Association with BRG1**—In contrast to the repressive activity of the carboxyl-terminal region of Mi-2β, we found that the amino-terminal region (1–376 amino acids; see fragment A in Fig. 4A) of Mi-2β has a strong transcriptional activating ability, resulting in a 10–20-fold increase of luciferase activity (Fig. 6A). When shorter fragments (fragments B and C in Fig. 4A) were used for the assay, the transactivating ability markedly decreased (Fig. 6A), indicating that the whole region of fragment A is necessary for transactivation. To elucidate whether binding of other proteins regulates the transactivating ability of the amino-terminal region of Mi-2β, we performed the immunoprecipitation experiments using human embryonic kidney 293 cells. First, the FLAG expression vectors containing three different regions of Mi-2β were transfected into the cells, and the resulting lysates were immunoprecipitated with an anti-FLAG monoclonal antibody, followed by immunoblotting with an anti-BRG1 antibody. As shown in Fig. 7A, BRG1 was specifically co-immunoprecipitated with the amino-terminal region of Mi-2β, whereas three other fragments examined (A, C, and D) showed no binding activity (Fig. 6C). These findings suggested that the unique transcriptional activating ability of the amino-terminal region of Mi-2β may be regulated by direct association with the bromodomains of BRG1.

To investigate the association of BRG1 with Mi-2β in vivo, we performed the immunoprecipitation experiments using human embryonic kidney 293 cells. First, the FLAG expression vectors containing three different regions of Mi-2β were transfected into the cells, and the resulting lysates were immunoprecipitated with an anti-FLAG monoclonal antibody, followed by immunoblotting with an anti-BRG1 antibody. As shown in Fig. 7B, BRG1 was specifically co-immunoprecipitated with the amino-terminal region of Mi-2β, whereas three other fragments examined (A, C, and D) showed no binding activity (Fig. 6C). These findings suggested that the unique transcriptional activating ability of the amino-terminal region of Mi-2β may be regulated by direct association with the bromodomains of BRG1.

The association between the carboxyl-terminal region of BRG1 and the amino-terminal region of Mi-2β (1–376 amino acids) was investigated by a yeast two-hybrid assay. As a consequence, a positive association was detected between the bromodomain of BRG1 (fragment B in Fig. 6B) and Mi-2β, whereas three other fragments examined (A, C, and D) showed no binding activity (Fig. 6C). These findings suggested that the unique transcriptional activating ability of the amino-terminal region of Mi-2β may be regulated by direct association with the bromodomains of BRG1.

**Involvement of BRG1 in the Transactivating Ability of Mi-2β**—Finally, we assessed the effect of BRG1 on the trans-
Association of Mi-2β with RFP and BRG1

Mi-2 has chromatin remodeling activity and forms a protein complex involved in epigenetic transcriptional repression. Recent reports (31–35) suggest the cooperation of Mi-2, Polycomb proteins, HDAC activity, and methyltransferase activity in transcriptional repression. In fact, it was shown that human Polycomb group protein EED interacts with HDAC (31) and that Drosophila dMi-2 functions in Polycomb group gene silencing (13). In addition, KAP-1, a member of the RBCC protein family, associates with Mi-2α (11).

RFP belongs to the RBCC-B30.2 protein family. These proteins contain a RING finger, a B-box zinc finger, a coiled-coil domain, and a specific carboxyl-terminal domain that was named the RFP domain or the B30.2-like domain (15). To date, over 200 members of the RING finger protein family have been identified. These proteins are known to play diverse roles in oncogenesis, apoptosis, development, and ubiquitination (36–38). Both the RING finger itself and the RBCC motifs are suspected to be involved in protein-protein interactions responsible for various biological events (38). In our previous report (18), we showed that RFP is a transcriptional repressor and that its coiled-coil domain directly associates with a Polycomb group protein, EPC1, suggesting that RFP may function physiologically in a protein complex formed by Polycomb group proteins. In the current study, we show that Mi-2β was also identified as a protein that interacts with RFP and enhances RFP-mediated transcriptional repression. The association of RFP and Mi-2β was further confirmed using Rfp−/− fibroblasts. These findings suggest that Mi-2 is involved in gene silencing that is mediated by a variety of RBCC proteins, as well as by Polycomb group proteins.

Mi-2 is an ATP-dependent nucleosome remodeling protein. Nucleosome remodeling domains have been identified in both transcriptional activating and repressing proteins, such as SWI/SNF-related proteins and Mi-2. Our present results indicate that the ATPase/helicase region of Mi-2 has little transcriptional activity, as indicated by the assay using all four luciferase constructs. Although nucleosome remodeling is probably a general property of epigenetic transcriptional control, we speculate that specific associations with these proteins may determine the consequence of the remodeling (39). Mi-2 forms a protein complex with HDAC and methyl CpG-binding protein in the transcriptional repressing NuRD protein complex (7, 8, 10). Mi-2 also binds several types of transcriptional repressors, including hunchback, Trk69, and KAP-1 co-repressor. Furthermore, its carboxyl-terminal region was identified as the binding site of their repression domains (11–13). In this study, we demonstrated that the Mi-2β carboxyl-terminal region interacts with the coiled-coil domain of RFP. Although the mechanism that makes this area repressive is unknown, we speculate that cooperation among various repressors and co-repressors is involved in the repressive activity of this region. In addition,
RFP, Mi-2, and HDAC were associated with each other and co-localized in the nucleus, suggesting the possibility that the function of RFP may also be linked with the chromatin remodeling and histone deacetylase activities. The transcriptional repressive activities of both full-length Mi-2β and its carboxyl-terminal region were almost abolished in the fibroblasts generated from a Rfp knockout mouse. This demonstrates the functional importance of the association of RFP with Mi-2 in RFP-expressing cells.

Surprisingly, we discovered that the amino-terminal region of Mi-2β has a strong transcriptional activating ability, in contrast to the repressing activity of its carboxyl-terminal region. The transcriptional repressive activities of both full-length Mi-2β and its carboxyl-terminal region were almost abolished in the fibroblasts generated from a Rfp knockout mouse. This demonstrates the functional importance of the association of RFP with Mi-2 in RFP-expressing cells.

Association of Mi-2β with RFP and BRG1

BRG1 is part of the SWI/SNF protein complex that is generally involved in transcriptional activation (42). BRG1 enhances the activity of the β-catenin and Tcf-target genes (28) and is required for the activation of Rb (43). However, interestingly, recent reports (22, 23) indicate that BRG1 also acts as a transcriptional repressor in various species including Drosophila, yeast, and mammals. For example, the interaction of BRG1 with HP1 modulated the heterochromatin structure and enhanced transcriptional repression (24). In addition, BRG1 was associated with Sin3A and was involved in transcriptional repression, especially in the repression of neural genes, by the

RFP and Mi-2 cooperate in transcriptional repression. A, transcriptional activities of Mi-2β in Rfp fibroblasts. The indicated regions of Mi-2β were cloned into the GAL4 DBD expression plasmid and transfected, along with the luciferase reporter plasmid containing the SRE enhancer and the SV40 promoter, into Rfp fibroblasts. The pFLAG-RFP expression plasmid (RFP, hatched) or a control plasmid (FLAG, black) were co-transfected (left panel). The lysates from Rfp fibroblasts untransfected (−) or transfected (+) with the pFLAG-RFP expression plasmid were analyzed by Western blotting with an anti-RFP antibody (right panel). Luciferase activities were measured as described. The amounts of plasmids used are given under "Experimental Procedures." IB, immunoblotting. B, enhancement of the RFP-mediated transcriptional repression by Mi-2β. The GAL4 DBD-RFP construct and the luciferase reporter plasmid containing the SRE enhancer and the SV40 promoter were co-transfected into 293 cells with increasing quantities (0, 100, 200, and 500 ng) of the full-length Mi-2β expression plasmid. Luciferase activities in the cells transfected with the GAL4 DBD-RFP construct were expressed as percentages of the control value. Each value represents the result of at least three experiments.
REST transcription factor (44, 45), indicating that the function of BRG1 is not limited to transactivation.

Whereas BRG1 and Mi-2 are the main subunits of SWI/SNF and NuRD, which are two well known chromatin remodeling complexes, BRG1 and Mi-2 could also be components of other multiprotein complexes in the context of transcriptional regulation, especially in development. In this regard, it is noteworthy that the Ikaros-containing chromatin remodeling complex (PYR complex) contains components of both the SWI/SNF and NuRD complexes in a single complex that includes Mi-2 and BRG1. It has been speculated that this complex plays a role in /H9253- to /H9252-globin switching in hematopoietic cells (29). In addition, ALL-1, a member of the human trithrax group protein family and a mammalian homologue of Drosophila trithorax, assembles the stable multiprotein supercomplex composed of over 29 proteins. These include the components of TFIID, SWI/SNF, NuRD, SNF2H, and Sin3A, and this complex binds the promoter region of the Hox a9 gene. Mi-2 and Brahma, which is highly homologous to BRG-1, are found to be involved in the ALL-1-containing multiprotein supercomplex (30). We speculate that direct association of chromatin remodeling proteins plays a key role in the formation of multiprotein complexes that includes components of distinct protein complexes such as SWI/SNF and NuRD.

In conclusion, we have identified transcriptional activating and repressing regions in the chromatin remodeling protein Mi-2β. These regions were associated with different proteins, BRG1 and RFP, and had distinct transcriptional activities. These findings provide new insight into the cooperation of transactivating and repressing protein complexes and the formation of multiprotein supercomplexes that are involved in transcriptional regulation.
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