A Novel Human Striated Muscle RING Zinc Finger Protein, SMRZ, Interacts with SMT3b via Its RING Domain*

Ken-Shwo Dai‡ and Choong-Chin Liew§

From the Institute of Medical Science and Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario MSG 1L5, Canada

The RING domain is a conserved zinc finger motif, which serves as a protein-protein interaction interface. Searches of a human heart expressed sequence tag data base for genes encoding the RING domain identified a novel cDNA, named striated muscle RING zinc finger protein (SMRZ). The SMRZ cDNA is 1.9 kilobase pairs in length and encodes a polypeptide of 288 amino acid residues; analysis of the peptide sequence demonstrated an N-terminal RING domain. Fluorescence in situ hybridization localized SMRZ to chromosome 1p33–34. Northern blots demonstrated that SMRZ is expressed exclusively in striated muscle. In the cardiovascular system, SMRZ is more highly expressed in the fetal heart than in the adult heart (slightly higher expression in the ventricle than in the atrium), suggesting that SMRZ is developmentally regulated. SMRZ was found to interact with SMT3b, a ubiquitin-like protein, through the SMRZ-RING domain. This interaction was abolished by mutagenesis of conserved RING domain residues. Transient transfection of SMRZ into C2C12 myoblasts showed localization of SMRZ to the nucleus. These data suggest that SMRZ may play an important role in striated muscle cell embryonic development and perhaps in cell cycle regulation.

The zinc finger proteins (ZFPs)1 are a group of proteins containing zinc finger domains. The finger-like structures are formed by the coordination of conserved cysteines (C) and histidines (H) with zinc ions (1–3). The discovery of the zinc finger domain in the Xenopus transcription factor IIIA (4), together with ongoing cDNA sequencing projects, has led to a significant acceleration in the discovery of ZFP genes. Combinatorial diversities among the conserved cysteines and histidines of the domain classifies ZFPs into several types: C2H2, C2C2, C2HC, C2HC4C(HD), C3H, and C3HC4 (3, 5–12). These ZFP types have been described in many different species indicates that the domain is essential to basic cellular function and is conserved during evolution. With the aid of sequence alignment, the consensus sequence of this domain was defined as CX2XC9–29CX1–1HX2–3CX14–48CXJC, where X can be any amino acid. Unlike other zinc finger domains, this domain shows a unique cross-brace arrangement on the conserved residues that coordinate the two zinc ions (15, 16).

Accumulated evidence suggests that the RING domain is mainly involved in protein-protein interactions (15, 16), and the structural integrity of RING is reportedly essential for this type of interaction. For example, deletion of RING domain resulted in a loss of activity in Bmi-1 transformation (17) and in PML interaction with IE1 (18). The RING domain mutation was found in the tumor suppressor BRCA1 (19, 20), and has been reported to cause impairments in bmi-1, which induces lymphomas (21); in c-Cbl, which desensitizes epidermal growth factor receptor (22); in MSL2, which forms protein complexes (23); in Pex12p, which maintains normal biological function of peroxisomes (24); in JE2, which blocks cell cycle progression in S phase (25); and in Vmv110, which interacts with PML-containing nuclear structures (26).

In an effort to improve our understanding of the functional roles of ZFPs and the RING domain, we identified a novel RING domain ZFP gene, named SMRZ, through a human EST data base sequence similarity search. Northern expression analysis indicated that SMRZ shows restricted expression in striated muscle and its pattern of expression in heart tissue suggests that the gene is developmentally regulated. Fluorescence in situ hybridization located SMRZ on chromosome 1p33–34. A yeast two-hybrid screen demonstrated an interaction between SMRZ and SMT3b, a ubiquitin-like protein. This interaction was confirmed using in vitro interaction assays. Site-directed mutagenesis analysis further strengthens the hypothesis that RING domain plays an essential role in protein-protein (SMRZ-SMT3b) interaction. Subcellular localization analysis showed that SMRZ is located in the nucleus.

**Experimental Procedures**

Isolation of cDNA Clone—An EST clone, named striated muscle RING zinc finger protein (SMRZ), exhibiting an amino acid sequence similar to the conserved RING domain sequence, was isolated from a human heart EST data base (27–29) using BLAST searches (30–32). The clone was subsequently excised in vivo from the λZAP Express
vector using the ExAssist/XLOLR helper phage system (Stratagene). In brief, phagemid particles were excised by co-infecting XLI-1 BLUE MRF’ cells with ExAssist helper phage. The excised pBluescript phagemids were used to infect Escherichia coli XLOLR cells, which lack the amber suppressor necessary for ExAssist phage replication. Infected XLOLR cells were subjected to kanamycin selection. Resultant colonies contained the double-stranded phagemid vector with the cloned cDNA insert. A single colony was grown overnight in LB-kanamycin, and DNA was purified using a plasmid purification kit (Qiagen).

**Northern Blot Analysis**—The expression pattern of SMRZ was analyzed using Northern blot hybridization. An ~1.2-kb SMRZ cDNA was amplified by PCR using full open reading frame was amplified with PCR primers containing BonHI and Bpu1102I enzyme digestion sites and inserted into the pET9a vector in-frame to generate pET9a-SMRZ plasmid. For Southern blot hybridization, genomic DNA was isolated from human fetal hearts (10–12 weeks) and treated with DNase I using Trizol reagents (Life Technologies, Inc.).

**In Vitro Interaction Assay**—To generate epitope-tagged SMRZ and SMT3b, two prokaryotic expression vectors, pET27b (+) and pET9a (Novagen), were used. The full open reading frame of SMRZ was amplified with PCR primers containing EcoRI and SalI enzyme digestion sites and ligated into the corresponding sites of pET27b (+) vector in-frame to generate pET27b (+)SMRZ plasmid. For bacterial lysate analysis, His→6X-His fusion proteins were eluted using buffer containing 1 M imidazole, 500 mM NaCl, and 20 mM Tris (pH 7.0) to remove unbound HMV-SMRZ and His→6X-SMT3. Bacterial cell lysates from *E. coli* BL21 (DE3) expressing tSMRZ fusion protein were split into aliquots and incubated with His-Bind resin immobilized with HMV-SMRZ or HMV-SMRZ-Z’ in a final concentration of 1 ml ATP in 1.0 ml of lysis buffer (Bacterial Cell, Transfection, and Confocal Microscopy Analysis—). Following incubation at 4 °C overnight (with gentle rocking), the mixture was pelleted by centrifugation and washed three times with buffer containing 60 mM imidazole, 500 mM NaCl, and 20 mM Tris·HCl (pH 7.9). The bound proteins were eluted using buffer containing 1 M imidazole, 500 mM NaCl, and 20 mM Tris·HCl (pH 7.9). SDS sample buffer was added to the protein elution under reducing and non-reducing conditions for recombinant protein analysis using 12% SDS-polyacrylamide gel electrophoresis. Control experiments under reducing conditions were performed under conditions: 1) nontransformed bacterial lysates were used to incubate with His-Bind resin immobilized with HMV-SMRZ or HMV-SMRZ-Z’ and 2) bacterial lysates expressing tSMRZ were used to incubate with non-HMV-SMRZ immobilized His-Bind resin.

**Cell Culture, Transfection, and Confocal Microscopy Analysis**—C2C12 myoblasts were purchased from the American Type Culture Collection (ATCC) and maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (Life Technologies, Inc.) at 37 °C in a humidified 5% CO_2_ atmosphere. Before transfections, the full open reading frame of SMRZ was amplified with PCR primers containing EcoRI and XhoI enzyme digestion sites and ligated into the corresponding sites of pcDNA3.1 vector (Invitrogen) in-frame with V5 epitope-tag to generate pcDNA3.1-SMRZ plasmid. Sequence fidelity of this recombinant was verified by sequencing. The cells were plated on multiwell chamber slides (Nunc) 1 day before transfection at a density of 5 × 10^4_ cells/well. Transfections were carried out using LipofectAMINE Plus transfection reagent according to the manufacturer's instructions (Life Technologies, Inc.). Briefly, 0.7 μg of pcDNA3.1-SMRZ plus 1 μg of pCMV-hrGFP was transfected into C2C12 myoblasts using LipofectAMINE Plus transfection reagent in 50 μl of serum-free DMEM for 15 min at 37 °C. The complexes were then reconstituted with 2 μl of LipofectAMINE reagent in 50 μl of serum-free DMEM and incubated for 15 min at 37 °C. Complexes of DNA-plus-LipofectAMINE reagent were then added to each well. Three hours following transfection, medium containing the complexes was replaced with fresh medium. Forty-eight hours after incubation, the cells were fixed with 4%
paraformaldehyde in PBS for 15 min, permeabilized with 0.2% Triton X-100 in PBS for 5 min, blocked with 1% bovine serum albumin (blocking buffer) for 30 min, incubated with a 1:200 diluted alkaline phosphatase-conjugated anti-V5 monoclonal antibody (Invitrogen) in blocking buffer for 1 h, and stained with Vector® Red substrate (Vector Laboratories Inc.). After washing, the slides were analyzed using a confocal fluorescence microscope (Olympus). Cells transfected with pDNA3.1 vectors were used as control.

**Western Blot Analysis—**

Proteins separated by SDS-polyacrylamide gel electrophoresis were transferred to polyvinylidene difluoride membranes (Helix) in transfer buffer (25 mM Tris-HCl, pH 8.3, 192 mM glycine, 20% methanol) with a Mini Trans-Blot apparatus (Bio-Rad) for 1 h at 100 V. Proteins were immunoblotted with specific antibodies. For example, membrane blotted with purified T7SMT3b fusion protein and its co-immunoprecipitates was blocked with a 3% solution of nonfat milk powder in TBST buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.1% Tween 20) and incubated with 1:5000 dilution of T7 tag horseradish peroxidase conjugate (Novagen) at room temperature. Membrane blotted with purified HSVSMRZ or HSVSMRZMT fusion proteins and their co-immunoprecipitates was blocked with 3% solution of bovine serum albumin in TBST buffer, incubated with monoclonal antibody directed against HSV tag (dilution 1 into 10,000; Novagen) and then treated with 1:10,000 dilution of T7 tag horseradish peroxidase conjugate (Novagen) at room temperature. Membrane was washed with TBST for 5 × 1 min. Bound antibody was detected using ECL Western blotting detecting reagents as described by the manufacturer’s protocol (Novagen). The binding was visualized by exposing the blots to Kodak X-Omat film.

**RESULTS**

**Isolation and Sequence Analysis of SMRZ cDNA—**An EST clone (SMRZ; GenBank™ accession no. AF361946) exhibiting (approximately) 39% amino acid sequence similarity to the RING domain of several known ZFPs was isolated and sequenced. To obtain the full length of the cDNA, 5'-RACE was performed. The full-length nucleotide and predicted amino acid sequences of SMRZ are shown in Fig. 1. The 1903-bp cDNA contains an 864-bp open reading frame extending from 984 to 1847, which corresponds to an encoded protein of 288 amino acid residues with a predicted molecular mass of 32.3 kDa. The sequence around the initiation ATG codon at nucleotide 984–986 was matched with the Kozak consensus ((A/G)CCATGG) (36, 37). The sequence coding region contained a N-terminal RING domain between residues 2–67. In the 3'-untranslated region, a potential polyadenylation signal AATAAA (38) was detected 26 bases upstream of a poly(A) tail. A search of the EST data base, disclosed 10 ESTs (GenBank™ accession no. F35868, F21990, AI351421, F36292, F32077, F25523, F18452, C02645, F34432, and BE894237) showing identity to portions of the SMRZ coding region. Fifty ESTs also matched an Alu sequence containing portion (nucleotides 220–295) of the 5'-UTR. No gene sequence deposited in public data bases was found to match to SMRZ suggesting that SMRZ is a novel gene. A comparison of the amino acid sequence of the RING domain regions of SMRZ and 26 known RING domain-containing ZFPs is shown in Fig. 2. The alignment shows that the cysteine and histidine residues for constructing the RING finger (i.e., C3HC4) are 100% conserved (shown in bold). Six additional highly conserved (>50% conservation) amino acids within the sequences are also shown in the consensus sequence (CONS).

**Expression of SMRZ mRNA in Human Tissues—**To determine the expression of the SMRZ transcript in various human tissues, both human multiple-tissue and cardiovascular system Northern blot analyses were probed using DIG-labeled SMRZ cDNA. For the human 12-lane multiple-tissue blot, a single transcript of ~2.1 kb was detected exclusively in heart and
A Novel RING Zinc Finger Protein

Chromosomal Mapping of SMRZ Gene—Chromosomal localization of SMRZ was performed by FISH (Fig. 4). 4,6-Diamidino-2-phenylindole staining and detailed position analysis allowed for assignment of the signal to 1p33–34. No additional loci were detected under the conditions applied.

Yeast Two-hybrid Screening Reveals an SMRZ RING Domain-SMT3b Interaction—To determine whether the SMRZ-RING domain is the region involved in protein-protein interactions and to identify its possible interactive partners, a yeast two-hybrid screen was performed using two baits: the RING domain region of SMRZ and the non-RING domain region (a region outside of the RING domain) of SMRZ. Each bait was fused to GAL4 activation domain serving to screen a commercial human heart cDNA library fused to GAL4 DNA-binding domain. After screening 106 transformants, 12 selected positive clones were isolated from the bait fused with RING domain isolated from yeast Y190. The specificity of this interaction was strengthened by control experiments. For example, no T7SMT3b could be eluted with HIS3-SMRZ-immobilized His-Bind resin, bound proteins were eluted and analyzed under reducing (Fig. 5A) and non-reducing conditions (Fig. 5B). Under reducing conditions, the eluted proteins were detected as two bands corresponding to SMRZ and SMT3b, a ubiquitin-like protein. Thus, SMT3b was selected for further study as an interaction partner of SMRZ.

In Vitro Demonstration of Interaction between SMRZ and SMT3b—Yeast two-hybrid results demonstrated that RING domain is a protein-protein interface. This raises the question as to whether the full-length SMRZ protein can interact with SMT3b. Using bacterial cell lysate expressing T7SMT3b incubated with non-HSVSMRZ immobilized His-Bind resin, bound proteins were eluted and analyzed under reducing (Fig. 5A) and non-reducing conditions (Fig. 5B). Under reducing conditions, we examined the presence of this interaction under non-reducing conditions (Fig. 5B). A slight increase in the molecular masses of epitope-tagged proteins as compared with the predicted molecular weight of the protein, was due to the binding of T7SMT3b to the His-Bind resin. Further, no HSVSMRZ and T7SMT3b could be eluted from bacterial lysates expressing T7SMT3b incubated with non-HSVSMRZ-immobilized His-Bind resin, bound proteins were eluted and analyzed under reducing (Fig. 5A) and non-reducing conditions (Fig. 5B). A slight increase in the molecular masses of epitope-tagged proteins as compared with the predicted molecular weight of the protein, was due to the extra amino acids derived from the expression vectors, which primarily served as epitope tags (Fig. 5B, lanes 1 and 4). For example, the predicted molecular mass of SMRZ is 32.3 kDa. As compared with the predicted molecular weight of the protein, the slight increase in the molecular masses of

REFERENCES

---

FIG. 2. Alignment of the RING domain. A consensus sequence is presented at the bottom; gaps between conserved amino acids (>50% conservation) are indicated by dashes. Asterisks indicate upstream translated region (data not shown).

A Novel RING Zinc Finger Protein

---
epitope-tagged proteins was due to the extra amino acids (~7.26 kDa) derived from the expression vectors, which primarily served as epitope tags. Bands with higher molecular weight visualized by immunoblotting with anti-HSV antibody (Fig. 5B, lane 2) and anti-T7 antibody (Fig. 5B, lane 3) indicate that large protein complexes (HSVSMRZ-T7SMT3b) were formed. The purified, bacterially expressed HSVSMRZ and T7SMT3b fusion proteins used as controls (Fig. 5, A (lane 1) and B (lanes 1 and 4)) demonstrated that these proteins could be expressed as stable proteins.

The SMRZ RING Domain Is Necessary for Interaction with SMT3b—To further confirm that the RING domain is essential for HSVSMRZ-T7SMT3b interaction, site-directed mutagenesis of the RING domain was used for in vitro interaction analysis. The RING domain is known to form a unique cross-brace arrangement, with the first and the third pairs of conserved residues forming the first zinc binding site, and the second and the fourth pairs forming the second (15, 16). We substituted Cys26, His28, Cys31, and Cys34 of the RING domain with alanines, which disrupt the structural integrity of the RING domain. Under reducing conditions, no T7SMT3b could be detected (Fig. 6A, lane 4) suggesting that the interaction was
TABLE I
Selection of positive clones that interact with DNA binding domain in yeast two-hybrid system

| Construct | Plates with selective media | LacZ expression |
|-----------|-----------------------------|----------------|
| pAS2-R1   | +                           | +              |
| pAS2-R2   | +                           | +              |

Molecular mass standards are indicated in kDa.

<Figure 6> In vitro analysis of interaction between mutated SMRZ and SMT3b. Bacterial lysates expressing T7SMT3b fusion protein were incubated with His-Bind resin immobilized with HSVSMRZ. A, under reducing condition, no SMT3b could be detected using anti-T7 antibody (lane 3). B, under non-reducing condition, eluted from the incubation of bacterial lysates expressing T7SMT3b fusion protein and His-Bind resin immobilized with HSVSMRZ were analyzed by Western blot. Using anti-HSV antibody (left panel), no bound protein was detected as a large complex indicated by HSVSMRZ-T7SMT3b (lanes 2 and 3). Purified HSVSMRZ (lane 1) and T7SMT3b (lane 4) were used as control. Molecular mass standards are indicated in kDa.

<Figure 5> In vitro analysis of interaction between wild-type SMRZ and SMT3b. Bacterial lysates expressing T7SMT3b fusion protein were incubated with His-Bind resin immobilized with HSVSMRZ. A, under reducing condition, bound proteins were detected using both anti-HSV (lane 4, upper panel) and anti-T7 antibody (lane 4, lower panel). As a control, only SMRZ can be detected from elute obtained from non-transformed lysate incubated with His-Bind resin immobilized with HSVSMRZ (lane 3). No bands can be detected from the elute obtained from lysates expressing T7SMT3b that were incubated with His-Bind resin (lane 2). Both purified HSVSMRZ and T7SMT3b were obtained from affinity column and used as a molecular mass marker for comparison (lane 1). B, under non-reducing condition, elutes from the incubation of bacterial lysates expressing T7SMT3b fusion protein and His-Bind resin immobilized with HSVSMRZ were analyzed by Western blot. Using anti-HSV antibody (left panel) and anti-T7 antibody (right panel), no bound proteins was detected as a large complex indicated by HSVSMRZ-T7SMT3b (lanes 2 and 3). Purified HSVSMRZ (lane 1) and T7SMT3b (lane 4) were used as control. Molecular mass standards are indicated in kDa.

<Figure 7>

Abolished. This explanation was further strengthened by the observation that no higher molecular weight bands could be visualized by immunoblotting with anti-HSV antibody (Fig. 6B, lane 2) and anti-T7 antibody (Fig. 6B, lane 3) under non-reducing conditions, indicating that the large protein complexes (HSVSMRZ-T7SMT3b) were not formed. The purified mutated RING domain SMRZ (HSVSMRZ^-MT) used as a control (Fig. 6, A (lane 1) and B (lane 1)) demonstrated that HSVSMRZ^-MT was expressed as a stable protein in order to exclude the possibility that loss of the interaction in in vitro assay was due to the instability of the mutated protein. The interaction between SMRZ and SMT3b was completely abolished by the mutated SMRZ RING domain, indicating that the RING domain is essential to protein-protein interaction.

Subcellular Localization of SMRZ—To facilitate our understanding of the putative role of SMRZ, the subcellular localization of SMRZ was determined using a plasmid expression construct encoding a V5-tagged SMRZ (V5SMRZ) fusion protein transiently transfected into C2C12 myoblasts. Staining for V5SMRZ in transfected C2C12 cells was performed using alkanine phosphatase-labeled antibody against the V5 epitope tag and Vector Red substrate, which produced red fluorescent precipitate. Using confocal fluorescence microscope, red fluorescent spots were observed on the nucleus (Fig. 7A). In contrast, no such red spot was seen in control cells (Fig. 7B). It is thus clear that the expressed SMRZ fusion proteins are present in the nuclei.

DISCUSSION

The ZFPs are a group of proteins involved in a wide spectrum of cellular regulatory functions (13). Since protein-protein interaction plays an important role in cell regulatory networks, identification of novel ZFP genes and characterization of their protein-protein interactions will facilitate our understanding of ZFP cellular regulatory mechanisms. Previously, we have established a cardiovascular ZFP profile (cvbZFP; Ref. 13). To expand this cardiovascular ZFP profile, a sequence similarity analysis search of a human heart EST data base was carried out in order to identify novel ZFP genes. An EST clone (SMRZ) exhibiting an amino acid sequence similar to the RING domain, a protein-protein interaction RING domain (15, 16) was isolated and sequenced. With the aid of 5'-RACE, the full-length sequence of SMRZ was obtained. Analysis of the full-length sequence indicated that SMRZ contains an Alu sequence in the
nostaining using phosphatase-conjugated anti-V5 monoclonal antibody. Forty-eight hours after transfection, cells were fixed and immunostained using phosphatase-conjugated anti-V5 monoclonal antibody and Vector Red substrate. Subcellular localization of V5SMRZ was visualized by a confocal fluorescence microscope.

5′-UTR and a RING domain at the N terminus of the coding region. Examples of Alu sequence in the 5′-UTR have been shown by a survey of GenBank™ sequence data base, which indicated that 14% of the Alu-containing human cDNAs have Alu sequence located in the 5′-UTR (44). The role of Alu sequence in 5′-UTR is currently unknown although it is believed that the presence of Alu sequence may influence translation. The presence of the RING domain is of functional importance since this domain has been shown to be mainly involved in protein-protein interactions (15, 16).

RING was first described in the sequence of the human ring 1 gene (14) and was later found in a large number of proteins through sequence comparison analysis (15, 16). These proteins are involved in a variety of functions such as oncogenesis, signal transduction, peroxisome biogenesis, viral infection, development, transcriptional repression, and ubiquitination (15, 16). Since the RING domain serves as a protein-protein interface, we hypothesized that determination of the RING domain interaction partner may facilitate our understanding of the SMRZ regulatory role. Using a yeast two-hybrid screen, we found that the RING domain is the only region of SMRZ that is involved in protein-protein interaction, and we identified SMT3b as an interaction partner of SMRZ. The specificity of interaction between SMRZ and SMT3b and the essential role of SMRZ-RING domain in this interaction were further confirmed in vitro using bacterially expressed full-length SMT3b and wild-type or mutated SMRZ fusion proteins. These results are supported by previous studies demonstrating that the structural integrity of RING domain is critical for the protein-protein interactions of RING ZFPs (17–26).

The restricted expression of SMRZ mRNA in the human heart and skeletal muscle suggests that SMRZ plays an important role in striated muscle cells. SMRZ transcript is more highly expressed in human fetal heart than in adult heart, suggesting that SMRZ may be involved in the cardiovascular developmental process. It is interesting to note that ESTs matching to portions of SMRZ were mainly found in heart, muscle, and whole embryo libraries. A search of UniGene disclosed that these ESTs were located in UniGene cluster Hs.157119T, which was assigned to chromosome 1. This chromosomal assignment was supported by FISH analysis, which mapped SMRZ to chromosome 1p33–34, a region associated with cell proliferation and differentiation (45–54). This predicted putative role of SMRZ based on the chromosomal assignment was supported by the subcellular localization experiment, which showed that SMRZ is located in the nucleus using C2C12 myoblasts transfected with SMRZ.

To facilitate our understanding of the putative functional role of SMRZ, it is necessary to understand the biological activities of its interactive partner SMT3b, a member of the ubiquitin-like gene family. SMT3b was originally cloned as a human homolog to yeast Smt3, first isolated as a high copy mutation suppressor in Mif2 (55). MIF2 is an essential centromere protein binding to the A+T-rich CDEII region of centromere DNA (55). Loss of MIF2 function results in blocking of DNA replication and nuclear division (56), chromosome missegregation, mitotic delay, and aberrant microtubule morphologies (57). Although the functions of most ubiquitin-like proteins are still largely unknown, it is assumed that since members of the family share a significant degree of sequence similarity they may have similar functional roles. Putative roles for a few ubiquitin-like proteins have been proposed. For example, sentrin-1/PIC1/SUMO-1, a ubiquitin-like protein with high sequence homology to SMT3b (58), has been shown to interact with RING domain of PML, a tumor suppressor associated with the pathogenesis of acute promyelocytic leukemia (41, 42). Overexpression of Sentrin-1/PIC1/SUMO-1 has also been shown to protect cells from Fas/APO- and TNF-induced apoptosis (59). The interaction between Sentrin-1/PIC1/ SUMO-1 and PML has been shown to be associated with cell cycle regulation (60–62). One recent study indicated that SMT3b may play a role in cellular responses to environmental stress, as SMT3b was shown to be up-regulated by many protein-damaging stimuli (63). In addition, SMT3P1, a novel isopeptidase 1 specifically bound to SMT3b, was found to be located in the nucleolus at interphase (64). These data, together with our Northern blot analysis, the chromosomal assignment, and the subcellular localization of SMRZ, leads us to believe that SMRZ-SMT3b interaction may be involved in cell cycle regulation occurring during the developmental process of striated muscle cells. Further study is needed to develop this interpretation.

In conclusion, we discovered a novel RING ZFP (SMRZ), analyzed its full-length sequence, and mapped its expression patterns, chromosomal, and subcellular locations. The identification of an interaction between SMRZ RING domain and SMT3b, as demonstrated in our yeast two-hybrid screen, in vitro interaction analysis, and site-directed mutagenesis, provides evidence indicating that SMRZ-RING domain plays an important role in protein-protein interaction and suggests that this interaction may be involved in the cell cycle regulatory process of striated muscle cells.

REFERENCES

1. Klug, A., and Rhodes, D. (1987) Trends Biochem. Sci 12, 464–469
2. el-Baradi, T., and Pieler, T. (1991) Mech. Dev 35, 155–169
3. Klug, A., and Schwabe, J. W. (1995) FASEB J. 9, 597–604
4. Miller, J., McLachlan, A. D., and Klug, A. (1985) EMBO J. 4, 1609–1614
5. Covey, S. N. (1986) Nucleic Acids Res. 14, 623–633
6. Mazen, A., Menissier-de Murcia, J., Molinet, M., Simonin, F., Gradwohl, G., Poirier, G., and de Murcia, G. (1989) Nucleic Acids Res. 17, 4689–4698
7. DuBois, R. N., McLane, M. W., Ryder, K., Lau, L. F., and Nathans, D. (1990) J. Biol. Chem. 265, 19185–19191
8. Freyf, G., Kim, S. K., and Horvitz, H. R. (1990) Nature 344, 876–879
9. Schwabe, W. R., and Rhodes, D. (1991) Trends Biochem. Sci 16, 291–296
10. Freemont, P. S. (1993) Ann. N. Y. Acad. Sci. 684, 174–192
11. O’Connor, J. G., Clere, G. M., Schaaf, O., Felsenfeld, G., Trainor, C., Appella, E., Stahl, S. J., and Grenenborn, A. M. (1993) Science 261, 438–446
