SRp55 Is a Regulator of Calcitonin/CGRP Alternative RNA Splicing†

Quincy Tran and James R. Roesser*

Department of Biochemistry, Virginia Commonwealth University, 1101 East Marshall Street, Richmond, Virginia 23298

Received August 28, 2002; Revised Manuscript Received November 21, 2002

ABSTRACT: Alternative splicing is an important mechanism for the regulation of gene expression. The mammalian calcitonin/calcitonin gene-related peptide (CGRP) pre-mRNA is alternatively spliced in a tissue-specific manner, leading to the production of calcitonin mRNA containing exons 1−4 in thyroid C cells and CGRP mRNA containing exons 1−3, 5, and 6 in neurons. The calcitonin-specific fourth exon contains an exonic splice enhancer (ESE) that binds SRp55. We define the RNA binding site of SRp55 in the ESE and demonstrate that base changes that decrease the level of SRp55 binding decrease the level of calcitonin splicing in vitro and calcitonin mRNA production in vivo. Base changes that increase the affinity of SRp55 for the ESE increase the level of calcitonin splicing in vitro and calcitonin mRNA levels in 293 cells. We also observe that SRp55 levels in different cell types correlate with the levels of calcitonin mRNA produced in these cells. Finally, we show that increasing the level of cellular expression of SRp55 stimulates calcitonin mRNA production in vivo. These observations suggest that SRp55 binding to a suboptimal RNA binding site in the calcitonin/CGRP pre-mRNA ESE is required for calcitonin mRNA production. Differential amounts of SRp55 present in different cell types would then control calcitonin/CGRP alternative splicing.

Alternative RNA splicing is an important mechanism for increasing proteomic complexity, allowing the production of multiple, functionally distinct proteins from a single gene (*1*, *2*). Moreover, alternative splicing is a crucial method for control of tissue-specific expression of related proteins. The mechanisms that control alternative mRNA splicing, however, are poorly understood.

The calcitonin/calcitonin gene-related peptide (CGRP)[1] gene is a model gene for the study of tissue-specific alternative RNA processing. The calcitonin/CGRP gene has six exons, and splicing together the first four exons generates calcitonin mRNA, which encodes the serum calcium regulating hormone calcitonin (Figure 1A). In thyroid c cells, more than 98% of the mature mRNA derived from the calcitonin/CGRP gene encodes calcitonin (*3*). Alternatively, more than 95% of the transcripts from the calcitonin/CGRP gene in neurons are processed into CGRP mRNA containing exons 1−3, 5, and 6 (*4*). Processing of the calcitonin/CGRP pre-mRNA is regulated by multiple elements at the level of both polyadenylation and alternative splicing (*5*−*11*).

An exonic splice enhancer (ESE) in calcitonin-specific exon 4 is required for incorporation of exon 4 into mRNA both in vitro and in vivo (*10*, *11*). This calcitonin-specific ESE consists of two parts, A and B elements. Results from this laboratory have shown that human transformer 2β (hTra2β) binds to A element RNA. SRp55, a member of the SR family of splicing factors, was the only protein in HeLa nuclear extract found to specifically cross-link to B element RNA after UV irradiation (*12*). Expression of SRp55 at different levels in different cell types (*13*) may thus potentially regulate tissue-specific alternative splicing of the calcitonin/CGRP pre-mRNA.

EXPERIMENTAL PROCEDURES

Plasmid Construction. Plasmid $\Delta\beta$1−4 was described previously (*14*). $\Delta\beta$1−4 match was created by oligonucleotide-directed mutagenesis using $\Delta\beta$1−4 DNA as a template and the oligonucleotides 5′ATTGGGGTTGGTCATCCTGGCAAGAAAA3′ and 5′TTTTCTTGCCAGGATGACCAACCCCAAT3′ (base changes underlined). $\Delta\beta$1−4 mismatch was constructed by directed mutagenesis using $\Delta\beta$1−4 DNA as a template and the oligonucleotides 5′ATTGGGGTTGGGCAGCTTATCCCAAGAAAA3′ and 5′TTTTCTTGGGATAAGCTGCCCAACCCCAAT3′ (base changes underlined).

DNA containing the B element was amplified by PCR using the primers 5′CCCGCATGCTTCAATTGGGGTTGGAG3′ and T3 primer 5′AATTAACCCTCACTAAAGGG3′ using ΔAcceptor 4 (*14*) as the template. The engineered *Sph*I site has been underlined. The PCR product was digested with *Sph*I and *Bam*HI and ligated into pGEM4Z (Invitrogen) cut with the same enzymes to make the pGEM4Z B element. B elements containing the SRp55 match and SRp55 mismatch mutations were amplified by PCR using $\Delta\beta$1−4 match or $\Delta\beta$1−4 mismatch DNA and oligonucleotide 5′TTCAACTCGAGTTGGGCTCATCCTGGC3′ (introduced *Xho*I restriction site underlined) and an oligonucleotide complementary to the T7 promoter site in the polylinker as primers. The

† This work was supported by Grant J-498 from the Jeffress Foundation.

* To whom correspondence should be addressed. Phone: (804) 828-7755. Fax: (804) 828-1473. E-mail: jroesser@hsc.vcu.edu.

[1] Abbreviations: CGRP, calcitonin gene-related peptide; mRNA, messenger RNA; ESE, exonic splice enhancer; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; EMSA, electrophoretic mobility shift assay; hTra2β, human transformer 2β.

10.1021/bi026753a CCC: $25.00
Published on Web 01/04/2003

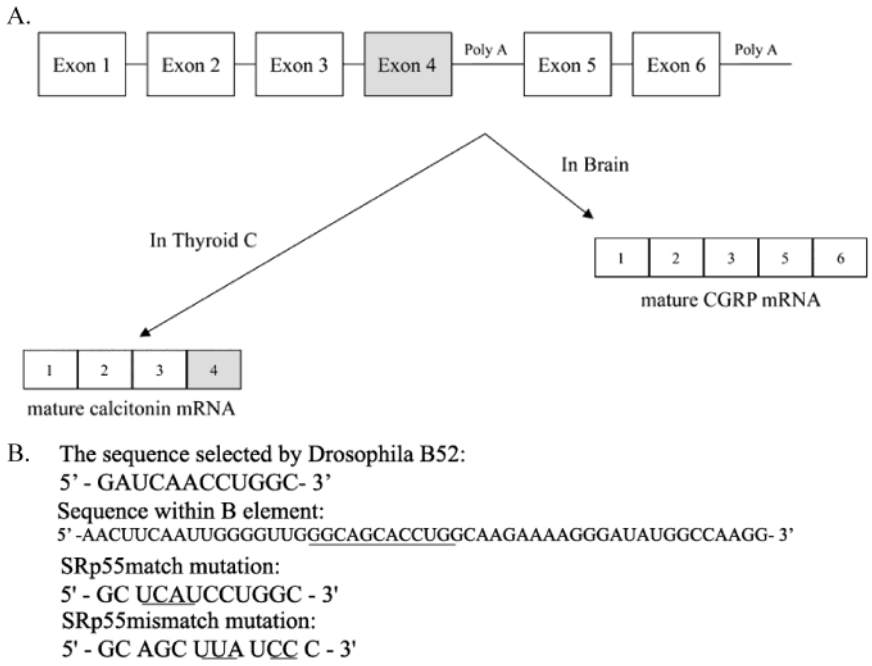


FIGURE 1: (A) Processing of the calcitonin/CGRP gene. Structure of the calcitonin/CGRP gene. Boxes denote exons. (B) B52 selected consensus binding sequence and sequences of the wild-type B element of the rat calcitonin-specific ESE and the SRp55 match and SRp55 mismatch mutations. The sequence in the B element resembling the B52 selected site and base changes at the site are underlined.

PCR products were digested with *Xho*I and *Bam*HI restriction endonucleases and ligated into pBKS (Stratagene) cut with the same enzymes to make pBKS B element SRp55 match plasmid and pBKS B element SRp55 mismatch.

To introduce mutations into the full-length rat calcitonin/CGRP gene, oligonucleotide-directed PCR mutagenesis was performed using the *Apa*I fragment of calcitonin gene in pBKS as a template. *Apa*I fragments containing the base changes were then ligated into HSRα calcitonin/CGRP (*9*) cleaved with *Apa*I. Primers 5′CCCAAACTTCAATTGGGGTTGGTCATCCTGGCAAGAAAAGG and 5′GGCCATATCCCTTTTCTTGCCAGGATGACCAACCCCAATTGAAGTTTGGG (base changes underlined) were used to construct calcitonin/CGRP SRp55 match. 5′CCCAAACTTCAATTGGGGTTGGGCAGCTTATCCCAAGAAAAGGGA3′ and 5′GGCCATATCCCTTTTCTTGGGATAAGCTCCAACCCCAATTGAAGTTTGGG3′ were used as primers to make HSRα calcitonin/CGRP SRp55 mismatch.

RNA Synthesis. Radiolabeled B element match RNA, B element mismatch RNA, Δβ1−4 RNA, Δβ1−4 SRp55 match RNA, and Δβ1−4 SRp55 mismatch RNA were synthesized by T3 RNA polymerase in the presence of [α-^{32}P]UTP (800 Ci/mmol) using the appropriate plasmid digested with *Bam*HI as a template. B element RNA was transcribed by T7 RNA polymerase in the presence of [α-^{32}P]-UTP with pGEM4Z B element DNA digested with *Bam*HI as a template.

In Vitro Reactions. UV cross-linking and gel mobility shift assays were performed as previously described (*9*). In vitro splicing reactions were carried out as described previously (*9*) except that reaction mixtures contained either 30 or 50 vol % HeLa nuclear extract.

In Vivo Analysis. Plasmids were introduced into 293 cells with lipofectamine plus reagent (Invitrogen). RNA was harvested 48 h after transfection by the addition of 1 mL of TRIzol reagent (Invitrogen). RNA was isolated and stored according to the manufacturer's instructions (Invitrogen).

Total RNA was isolated and analyzed for splice products using RT-PCR. RT-PCR was performed essentially as described previously (*14*). All RT-PCRs were performed in the linear range; using 30 amplification cycles, the PCR products were extracted, separated by electrophoresis on agarose gels and visualized by staining with ethidium bromide. The sequence of the primer complementary to calcitonin exon 4 used in PCR amplification was 5′CTTGTTGAGGTCTTGTGTGTA3′. The sequence of the second primer complementary to exon 3 was 5′CCTACTGGCTGCACTGGTGC3′, and the sequence of the primer complementary to exon 5 was 5′TTCTTTCTGGGGCTATTATC3′.

To examine the expression of cotransfected SRp55 cDNA, cDNAs from the transfected cells were amplified using Taq Polymerase and T7 primer 5′GTAATACGACTCACTATAGGGC3′ (T7 site is in the 5′ untranslated region and is derived from the pCR3 vector) and SRp55-specific primer 5′TTAATCTCGGAACTCGACC3′.

SR Protein Analysis. Nuclear extracts were prepared as described previously (*15*). Five micrograms of nuclear proteins was separated by SDS−PAGE on 12% polyacrylamide gels and electroblotted onto nitrocellulose membranes. Membranes were probed with mAb104 (*16*) (1:50 dilution) and visualized by chemiluminescence.

Expression of SRp55. The cDNA for human SRp55 was isolated from HeLa cells. HeLa cell RNA was converted to cDNA with thermoscript reverse transcriptase (Invitrogen) using random hexamer primers. Primers 5′CGCGGATCCATGCCGCGCGTCTACATAG3′ and 5′CGCGAATTCTTAATCTCTGGAACTCGACC3′ were used to amplify SRp55 in the presence of Taq polymerase. The PCR product was cut with *Eco*RI and *Bam*HI and ligated downstream of the CMV promoter in pCR3 (Invitrogen) cut with the same enzymes.

For expression of SRp55 as a GST fusion protein, HeLa cell RNA was converted to cDNA with thermoscript reverse

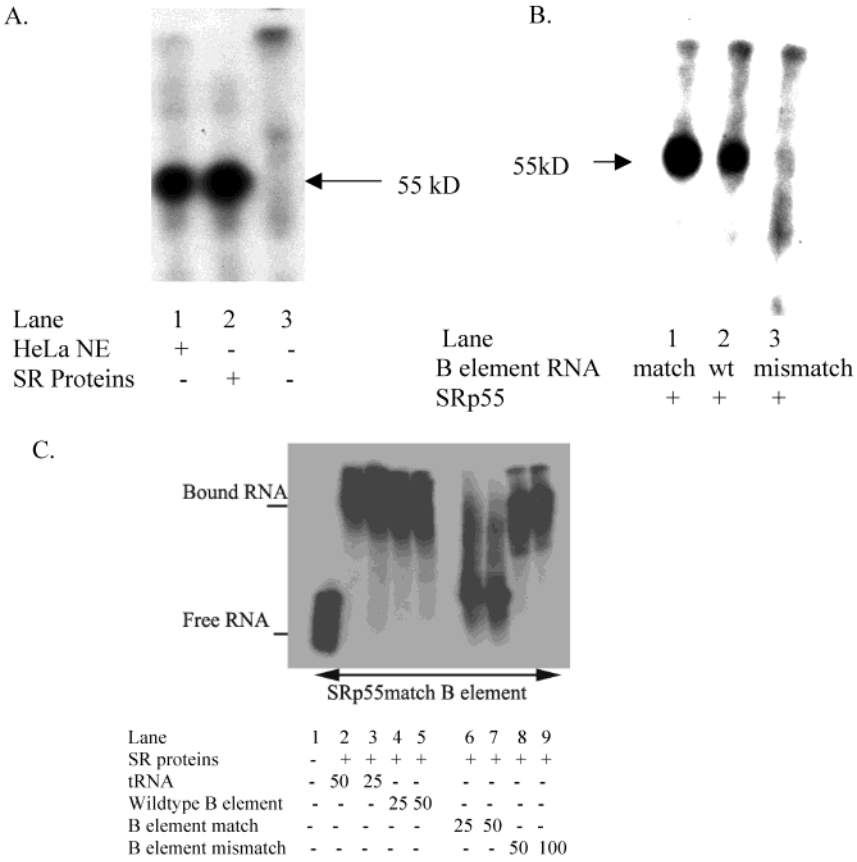


FIGURE 2: SRp55 binds to a site in the calcitonin-specific ESE that resembles a B52 binding site. (A) UV cross-linking of uniformly ^{32}P-radiolabeled wild-type B element RNA. Substrate RNA was incubated with HeLa nuclear extract (5 μg of protein, lane 1), purified SR proteins (20 of ng, lane 2), or no protein (lane 3). A 200-fold molar excess of unlabeled tRNA was included during the incubation. Protein−RNA complexes were irradiated with 254 nm light. After treatment with RNAse A, reactions were analyzed by electrophoresis on 10% polyacrylamide−0.1% SDS gels. RNA was visualized by autoradiography. (B) UV cross-linking of uniformly ^{32}P-radiolabeled wild-type B element RNA (lane 2), radiolabeled B element SRp55 mismatch RNA (lane 3), or radiolabeled B element SRp55 match RNA (lane 1) with purified SRp55 protein. Ten nanograms of purified SRp55 protein and a 200-fold molar excess of unlabeled tRNA were incubated with substrate RNAs and assayed by UV cross-linking as described in panel A. (C) Radiolabeled B element SRp55 match RNA was incubated in the absence (lane 1) or presence of 20 ng of total SR proteins (*27*) and unlabeled competitor RNAs. Complex formation was analyzed by separation on 5% nondenaturing polyacrylamide gels. Complexes were visualized by autoradiography. The molar excesses of unlabeled competitor RNAs vs radiolabeled B element SRp55 match RNA are indicated.

transcriptase (Invitrogen) using random hexamer primers. Primers 5′CGCGGATCCATGCCGCGCGTCTACATAG3′ and 5′CGCGAATTCTTAATCTCTGGAACTCGACC3′ were used to amplify SRp55. The PCR product was cut with *Eco*RI and *Bam*HI and cloned into pGEX-6P-1 (Amersham). GST fusion proteins were expressed in *Escherichia coli* strain JM101 and purified as described previously (*17*). SRp55 was separated from GST by digestion with precission protease (Amersham).

RESULTS

Binding of SRp55 and hTra2β to the calcitonin-specific ESE suggests that binding of these factors may be required for promotion of calcitonin splicing by the ESE. To test this hypothesis, we sought to more precisely define the site of interaction between SRp55 and element B of the ESE. We decided to concentrate on the interaction of SRp55 with the B element as results from this laboratory indicated that the B element is absolutely required for exon 4 splicing, while the A element is only necessary when the B element is distant from the exon 4 splice acceptor (*12*). SRp55 is the only protein from HeLa nuclear extract or purified SR proteins that specifically binds B element RNA [Figure 2A (*12*)]. SRp55 binds preferentially to purine-rich ESEs present in several genes (*18*), and the B element contains a purine-rich region (Figure 1B). However, electrophoretic mobility shift assays (EMSAs) indicated that neither HeLa nuclear extract nor purified SR proteins formed complexes with RNA containing the purine-rich sequence from the B element. RNA containing the purine-rich region and the 10 nucleotides immediately upstream, however, formed specific complexes with HeLa nuclear extract (data not shown), suggesting that the purine-rich sequence alone was insufficient for SRp55 binding.

The RNA sequence 5′ of the purine-rich element resembles the preferred binding site for B52, the *Drosophila* homologue of SRp55 (Figure 1B) (*19*). To determine whether SRp55 interacts with this B52-like sequence, we made base changes in the sequence to make it a better match to the B52 selected sequence (SRp55 match) or changes that made it a poorer match to the B52 consensus binding site (SRp55 mismatch, Figure 1B). B element SRp55 mismatch RNA did not bind to purified SRp55 protein when assayed by UV cross-linking (Figure 2B, lane 3). Conversely, B element SRp55 match RNA binds to purified SRp55 in the presence of a 200-fold molar excess of tRNA (Figure 2B, lane 1). Formation of a

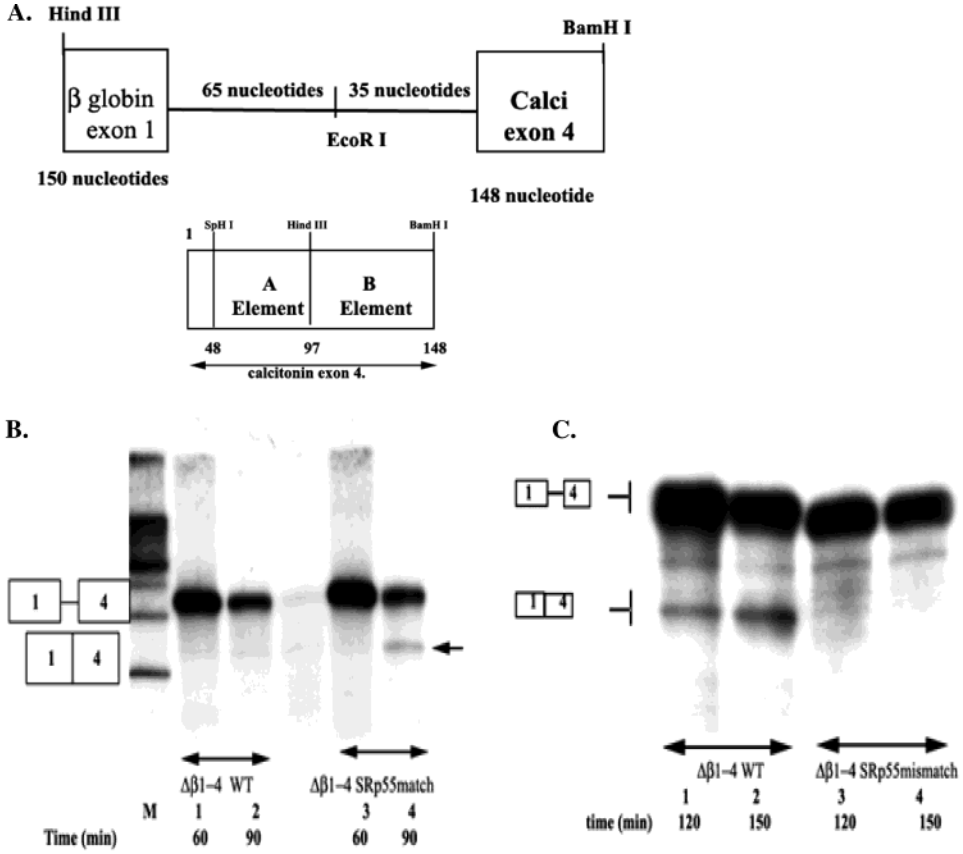
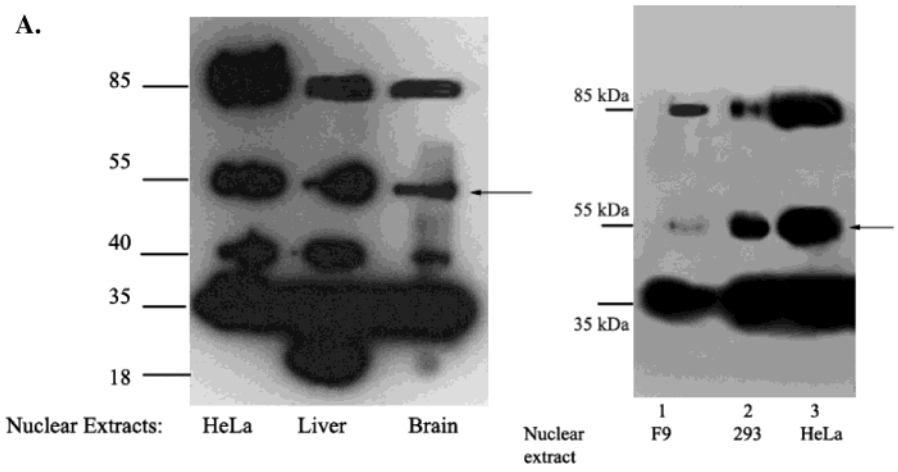


FIGURE 3: SRp55 binding to the calcitonin-specific ESE is required for calcitonin splicing in vitro. (A) Structure of the $\Delta\beta1-4$ minigene (*14*) and the calcitonin-specific ESE. (B) In vitro splicing of $\Delta\beta1-4$ and $\Delta\beta1-4$ SRp55 match RNAs. Uniformly radiolabeled $\Delta\beta1-4$ and $\Delta\beta1-4$ SRp55 match RNAs were incubated under splicing conditions. Splicing reaction mixtures contained 30% HeLa nuclear extract by volume. RNAs were separated by electrophoresis on 8% polyacrylamide−8 M urea gels, and products were visualized by autoradiography. An arrow indicates spliced $\beta1-4$ RNA. (C) In vitro splicing of $\Delta\beta1-4$ and $\Delta\beta1-4$ SRp55 mismatch RNAs was carried out as described for panel B, except splicing reaction mixtures contained 50% by volume HeLa nuclear extract.

complex between radiolabeled B element SRp55 match RNA and purified SR proteins was examined in the presence of unlabeled competitor RNAs by an EMSA. A 50-fold molar excess of tRNA, B element RNA, or B element SRp55 mismatch RNA had little effect on complex formation (Figure 2C, lanes 2, 5, and 8). A 25-fold molar excess of unlabeled B element SRp55 match RNA abolished complex formation (Figure 2C, lane 6), suggesting that SRp55 has a higher affinity for B element SRp55 match RNA than for wild-type B element RNA. Unlabeled B element SRp55 match RNA was also able to disrupt formation of a complex between B element RNA and SR protein preparations (data not shown). These observations strongly suggest that SRp55 binds to the B52 preferred site in calcitonin/CGRP pre-mRNA.

To test the hypothesis that SRp55 binding to the calcitonin-specific ESE controls calcitonin/CGRP RNA splicing, we prepared in vitro splice substrates containing the SRp55 match and SRp55 mismatch mutations. We have previously demonstrated that RNA from the chimeric minigene $\Delta\beta1-4$, which contains the human β-globin first exon and the first 65 nucleotides of intron 1 fused to the last 39 nucleotides of calcitonin/CGRP intron 3 and the first 148 nucleotides of exon 4 (Figure 3A), is accurately spliced in vitro (*14*). When used as splice substrates in HeLa nuclear extract, $\Delta\beta1-4$ SRp55 match RNA was spliced more efficiently than $\Delta\beta1-4$ RNA, while $\Delta\beta1-4$ mismatch was spliced much less efficiently (Figure 3B,C). These observations suggest that SRp55 binding to B element RNA is necessary for exon 4 splicing in vitro.

The observation that binding of SRp55 to the calcitonin-specific ESE may be required for calcitonin-specific splicing suggests that varying levels of SRp55 expression in different cell types could control tissue-specific calcitonin/CGRP splicing. To examine this possibility, the levels of SRp55 present in cell and tissue types where the splicing pattern of calcitonin/CGRP is known were determined by Western blot analysis of nuclear extracts using mAb104 as a probe for SR protein content. mAb104 specifically interacts with members of the SR family of splice regulators (*16*), and representative Western blots are shown in Figure 4A. Brain and F9 cells which produce predominantly CGRP mRNA (*20*, *21*) contain little SRp55 compared to liver or HeLa cells, which preferentially synthesize calcitonin mRNA (*20*, *21*). 293 cells produce nearly equal amounts of calcitonin and CGRP mRNA (*10*) and contain an intermediate amount of SRp55. These observations agree with a previous study that detected low levels of SRp55 in brain (*13*). Figure 4B summarizes the level of SRp55 we found in different cells and tissues. In support of the possibility that SRp55 levels play an important role in regulating calcitonin/CGRP splicing, cells with high SRp55 levels make predominantly



B.

	Normalized level of SRp55	Ratio of calcitonin/CGRP production
HeLa cells	89%	>98%
Liver Tissue	100%	>99%
293 cells	53%	60% calcitonin 40% CGRP
Brain Tissue	12%	<1%
F9 cells	9%	<1%

FIGURE 4: SRp55 levels in different cell types directly correlate with the levels of calcitonin mRNA production. (A) Western blot analysis of nuclear extract from HeLa, rat liver, rat brain, mouse F9, and human 293 cells. Five micrograms of nuclear extract proteins was separated by electrophoresis on 12% polyacrylamide gels containing 1% sodium dodecyl sulfate (SDS). Proteins were electroblotted onto nitrocellulose membranes for 1 h at 100 V. Membranes were washed and then incubated with mAb104 at a 1:50 dilution. Protein antibody interactions were visualized by chemiluminescence and the relative intensities of the bands determined by densitometry. Protein size markers (in kilodaltons) are shown, and arrows indicate SRp55. (B) SRp55 protein levels detected in the indicated cell types by Western analysis using mAb104. Amounts of SRp55 are expressed as a fraction of total SR proteins present in the cell and then normalized to the fraction of SRp55 in liver cells, which was arbitrarily set at 100. The percent of calcitonin mRNA of total processed calcitonin/CGRP RNA is indicated (*18*−*20*). Results were averages of at least three trials.

calcitonin mRNA. Conversely, cells with low levels of SRp55 preferentially produce CGRP mRNA, while cells with intermediate SRp55 concentrations make both calcitonin and CGRP mRNA.

To examine the effect of SRp55 on calcitonin/CGRP splicing in vivo, the SRp55 match and SRp55 mismatch mutations were introduced into the full-length rat calcitonin/CGRP gene. Mutant and wild-type genes were then transfected into 293 cells, and RNA isolated from transiently transfected cells was analyzed by reverse transcriptase and competitive PCR using primers complementary to calcitonin/CGRP exons 3−5. When expressed in 293 cells, the wild-type calcitonin/CGRP gene produces approximately 60% CGRP mRNA and 40% calcitonin mRNA (Figure 5B, lane 1). Calcitonin SRp55 mismatch pre-mRNA, however, which contains base changes that prevented SRp55 binding in vitro, is processed almost exclusively into CGRP mRNA (Figure 5B, lane 3). Calcitonin SRp55 match pre-mRNA, which contains base changes that increase the affinity of RNA for SRp55 in vitro, is spliced to produce more than 90% calcitonin mRNA (Figure 5B, lane 5).

Increasing the level of SRp55 expression by transfection of an SRp55 cDNA under control of the CMV promoter stimulated calcitonin mRNA production in 293 cells. Cotransfection of SRp55 cDNA with the wild-type calcitonin/CGRP gene changed the pattern of splicing from 40% calcitonin mRNA to more than 90% calcitonin mRNA (Figure 5B, lane 2). Expression of transfected SRp55 was followed by PCR analysis of cDNA from cotransfected cells using an SRp55-specific primer and a primer complementary to the 5′ untranslated region encoded by the expression vector (Figure 5B). Cotransfection of SRp55 cDNA with calcitonin SRp55 match or calcitonin SRp55 mismatch had little effect on splice choice (Figure 5B, lanes 4 and 6). This suggests that cellular SRp55 levels do not affect calcitonin/CGRP splicing when the SRp55−ESE interaction is very strong or very weak. This further suggests that suboptimal binding of SRp55 to the ESE may be important for the proper regulation of calcitonin/CGRP RNA splicing.

DISCUSSION

SRp55 has been shown to interact with a number of purine-rich ESEs to activate splicing of the ESE-containing exon (*18*, *22*, *23*). An ESE required for splicing of calcitonin exon 4 contains a purine-rich sequence, and SRp55 specifically binds to this ESE (*12*). SRp55 was the only protein from nuclear extract or purified SR protein preparations that specifically bound to B element RNA (*12*). We observe, however, that SRp55 does not interact with the purine-rich sequence in the calcitonin-specific ESE. Rather, SRp55 binds

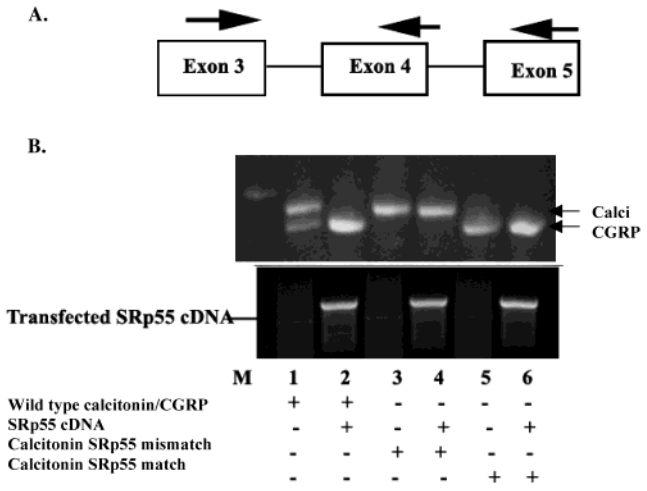


FIGURE 5: SRp55 binding controls calcitonin/CGRP pre-mRNA splicing in vivo. (A) Position of primers used for PCR amplification. (B) Calcitonin/CGRP genes under control of the HSRα (*9*) promoter were transfected into 293 cells. RNA from 293 cells was harvested 48 h after transfection. cDNA was synthesized from total cellular RNA by Thermoscript reverse transcriptase (Invitrogen) using random hexamer primers. Calcitonin/CGRP splice products were amplified using Taq polymerase and primers complementary to exons 3−5 for 30 amplification cycles. PCR products were separated on 2% agarose gels and visualized by ethidium bromide staining. Lane M contained DNA size markers. RNAs from cells transfected with the wild-type rat calcitonin/CGRP gene (lanes 1 and 2), calcitonin/CGRP mismatch (lanes 3 and 4), and calcitonin/CGRP match (lanes 5 and 6) are indicated. RNAs from cells cotransfected with SRp55 cDNA under control of the CMV promoter are in lanes 2, 4, and 6. Bands indicated as calcitonin and CGRP were excised from the gel, eluted, and subjected to DNA sequence analysis to confirm their identities. To examine the expression of cotransfected SRp55 cDNA, cDNAs from the transfected cells were amplified by PCR using a T7 primer (T7 site is in the 5′ untranslated region and is derived from the pCR3 vector) and SRp55-specific primer. PCR products were separated on a 1% agarose gel and visualized by ethidium bromide staining.

to a region adjacent to the purine tract that resembles an RNA binding site selected by B52, a *Drosophila* homologue of SRp55 (*19*). Synthetic RNAs containing the selected sequence specifically bind B52 (*24*), but no naturally occurring splice enhancers containing the B52 selected sequence have been previously described. The B52-like sequence in calcitonin exon 4 clearly functions as a splice enhancer because base changes at this site affect splicing of exon 4 in vitro and in vivo. It is not clear how SRp55 recognizes different types of RNA sites, but this behavior has been demonstrated in other RNA binding splice factors (*25*). SRp55 contains two RNA recognition motifs, which may allow it to recognize different RNA sequences. Alternatively, interactions with other proteins might alter the RNA binding affinity for SRp55.

We have demonstrated that base changes that allow more efficient binding of SRp55 to the calctonin-specific ESE, or increasing the amount of SRp55 in cells, stimulate calcitonin mRNA production. Alternatively, changes in the calcitonin-specific ESE that inhibit SRp55 binding also inhibit calcitonin mRNA production and increase CGRP mRNA levels. These observations demonstrate conclusively that SRp55 is an important regulator of calcitonin/CGRP alternative splicing and strongly suggest that binding of SRp55 to the calcitonin-specific ESE mediates this regulation. Moreover, our observations suggest that cellular levels of SRp55 play a crucial role in regulating tissue-specific alternative RNA splicing of the calcitonin/CGRP gene.

Processing of the calcitonin/CGRP pre-mRNA is regulated at the level of polyadenylation, as well as alternative splicing. A polyadenylation enhancer associated with the calcitonin-specific polyadenylation site in intron 4 is necessary for proper pre-mRNA processing (*5*, *6*). The enhancer acts as a pseudoexon that regulates calcitonin-specific polyadenylation. SRp20, U1 snRNP, and polypyrimidine tract binding protein (PTB) binding to the enhancer are required for proper processing of the pre-mRNA (*5*−*7*). Therefore, SRp55 is one of several regulatory factors known to interact with calcitonin/CGRP pre-mRNA to regulate processing of the RNA. The large change in the calcitonin mRNA:CGRP mRNA ratio observed when the level of expression of SRp55 is increased, or the SRp55 RNA binding site is altered, suggests that SRp55 plays a major role in controlling calcitonin/CGRP mRNA processing.

High levels of SRp55 were previously shown to favor usage of the distal 5′ splice site in the SV40 small t intron in vitro (*13*). In addition, overexpression of several SR proteins was demonstrated to modulate alternative splicing in vivo (*26*). Our experiments, however, provide the most direct evidence to date that naturally occurring differences in cellular levels of a splicing factor control the tissue-specific alternative splicing of a mammalian gene transcript.

ACKNOWLEDGMENT

We thank Dr. Carmen Sato-Bigbee and Dr. Timothy Coleman for helpful discussions.

REFERENCES

1. Gravely, B. (2001) *Trends Genet. 17*, 100−107.
2. Black, D. (2001) *Cell 103*, 367−370.
3. Sabate, M. I., Stolarsky, L., Polak, J. M., Bloom, S. R., Varndell, I. M., Ghatei, M. A., Evans, R. M., and Rosenfeld, M. G. (1985) *J. Biol. Chem. 260*, 2589−2592.
4. Amara, S. G., Jonas, V., Rosenfeld, M. G., Ong, E. S., and Evans, R. M. (1982) *Nature 298*, 240−244.
5. Lou, H., Yang, Y., Cote, G. J., Berget, S. M., and Gagel, R. F. (1995) *Mol. Biol. Cell 15*, 7135−7142.
6. Lou, H., Gagel, R. F., and Berget, S. M. (1996) *Genes Dev. 10*, 208−219.
7. Lou, H., Helfman, D. M., Gagel, R. F., and Berget, S. M. (1999) *Mol. Cell. Biol. 19*, 78−85.
8. Leff, S. E., Evans, R. M., and Rosenfeld, M. G. (1987) *Cell 48*, 517−524.
9. Roesser, J. R., Liittschwager, K., and Leff, S. E. (1993) *J. Biol. Chem. 268*, 8366−8375.
10. Van Oers, C. C., Adema, G. J., Zandberg, H., Moen, T. C., and Baas, P. D. (1994) *Mol. Cell. Biol. 14*, 951−960.
11. Zandberg, H., Moen, T. C., and Baas, P. D. (1995) *Nucleic Acids Res. 23*, 248−255.
12. Tran, Q., Coleman, T. P., and Roesser, J. (2002) *Biochim. Biophys. Acta* (in press).
13. Zahler, A. M., Neugebauer, K. M., Lane, W. S., and Roth, M. B. (1993) *Science 260*, 219−222.
14. Coleman, T. P., and Roesser, J. R. (1998) *Biochemistry 37*, 15941−15950.
15. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) *Nucleic Acids Res. 11*, 1475−1489.
16. Roth, M. B., Murphy, C., and Gall, J. G. (1990) *J. Cell Biol. 111*, 2217−2223.
17. Tacke, R., Tohyama, M., Ogawa, S., and Manley, J. L. (1998) *Cell 93*, 139−148.
18. Nagel, R. J., Lancaster, A. M., and Zahler, A. M. (1998) *RNA 4*, 11−23.

19. Shi, H., Hoffman, B. E., and Lis, J. T. (1997) *Mol. Cell. Biol. 17*, 2649−2657.
20. Yeakley, J. M., Hedjran, F., Morfin, J.-P., Merillat, N., Rosenfeld, M. G., and Emeson, R. B. (1993) *Mol. Biol. Cell 13*, 5999−6011.
21. Crenshaw, E. B., III, Russo, A. F., Swanson, L. W., and Rosenfeld, M. G. (1987) *Cell 49*, 389−398.
22. Lemaire, R., Winne, A., Sarkissian, M., and Lafyatis, R. (1999) *Eur. J. Immunol. 29*, 823−837.
23. Gontarek, R. R., and Derse, D. (1996) *Mol. Cell. Biol. 16*, 2325−2331.
24. Hoffman, B. E., and Lis, J. T. (2000) *Mol. Cell. Biol. 20*, 181−186.
25. Lynch, K. W., and Maniatis, T. (1996) *Genes Dev. 10*, 2089−2101.
26. Caceres, J. F., Stamm, S., Helfman, D. M., and Krainer, A. R. (1994) *Science 265*, 1706−1709.
27. Zahler, A. M., Lane, W. S., Stolk, J. A., and Roth, M. B. (1992) *Genes Dev. 6*, 837−847.

BI026753A