THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 276, No. 12, Issue of March 23, pp. 8665–8673, 2001

Printed in U.S.A.

The STAR/GSG Family Protein rSLM-2 Regulates the Selection of Alternative Splice Sites*

Received for publication, July 31, 2000, and in revised form, November 7, 2000
Published, JBC Papers in Press, December 15, 2000, DOI 10.1074/jbc.M006851200

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We identified the rat Sam68-like mammalian protein (rSLM-2), a member of the STAR (signal transduction and activation of RNA) protein family as a novel splicing regulatory protein. Using the yeast two-hybrid system, coimmunoprecipitations, and pull-down assays, we demonstrate that rSLM-2 interacts with various proteins involved in the regulation of alternative splicing, among them the serine/arginine-rich protein SRp30c, the splicing-associated factor YT521-B and the scaffold attachment factor B. rSLM-2 can influence the splicing pattern of the CD44v5, human transformer-2β and tau minigenes in cotransfection experiments. This effect can be reversed by rSLM-2-interacting proteins. Employing rSLM-2 deletion variants, gel mobility shift assays, and linker scan mutations of the CD44 minigene, we show that the rSLM-2-dependent inclusion of exon v5 of the CD44 pre-mRNA is dependent on a short purine-rich sequence. Because the related protein of rSLM-2, Sam68, is believed to play a role as an adapter protein during signal transduction, we postulate that rSLM-2 is a link between signal transduction pathways and pre-mRNA processing.

Prior to export to the cytosol, pre-mRNA generated from most eukaryotic genes undergoes maturation processes such as splicing, in which intronic sequences are removed and exonic sequences are rejoined, as well as polyadenylation and 5′-end capping. There is increasing evidence that transcription, pre-mRNA processing, and RNA transport are coupled in a highly coordinated manner (1–3). Recent results indicate a direct interaction among RNA polymerase II, transcription, capping, splicing, and polyadenylation factors (3–6). These complexes are possibly attached to chromatin, for example by the scaffold attachment factor B (SAF-B[1]) (7). This supports the model of a large RNA processing unit (8, 9) termed RNA factory (3). Pre-mRNA splicing is characterized by a high fidelity and can be modulated in a cell type- or development-specific way to use exons alternatively. Although the exact mechanisms governing splice site selection are still not fully understood, recent results indicate that loosely defined signals on the pre-mRNA known as splicing enhancers/silencers, play a crucial role in splice site selection (10–12). An important class of proteins that recognize splicing enhancers/silencers is the serine/arginine-rich (SR) and SR-related protein family that is involved in both constitutive and alternative splicing (13, 14). In addition, it has also been shown that an increasing number of heterogenous nuclear ribonucleoproteins (hnRNPs) are involved in the regulation of alternative splicing. For example, splicing regulation of the neuron-specific exon N1 or the *src* pre-mRNA is under the control of the hnRNPs hnRNP I (polypyrimidine tract binding protein), hnRNP F, and hnRNP H (15, 16).

SR proteins and hnRNPs can change alternative splicing patterns in a concentration-dependent manner both *in vivo* and *in vitro* (for review, see Refs. 13 and 14). Because the relative expression levels of SR proteins and hnRNPs show tissue-dependent variations (17–19), one hypothesis is that tissue-specific splicing is the result of concentration differences of ubiquitously expressed proteins that regulate the usage of a given splice site.

Another way of regulating alternative splicing decisions could be the presence of factors specific for a tissue type or developmental stage. A well known example is expression of the sex-specific SR-related protein transformer (20). In addition, tissue-specific proteins such as ELAV (embryonic lethal abnormal vision) (21) and NOVA (22) were described, and we previously found tissue-specific variants of the splice factor tra2-β (23) which have a different effect on a given splice site (24). Finally, several proteins involved in pre-mRNA splicing, judged to be ubiquitously expressed by Northern blot analysis, show cell type-specific expression when examined by histochemical methods. These include tra2-β1 (23, 25), hnRNP L (19), and YT521-B (26).

The nuclear RNA-binding protein Sam68 was first described as a target of the tyrosine kinase Src during mitosis (27, 28). Sam68 is a member of the STAR (signal transduction and activation of RNA) protein family (29), also called GSG protein family (GRP33, Sam68, GLD-1) (30, 31), whose members share a slightly extended hnRNP K homology RNA binding domain, called maxi-KH domain. Because Sam68 binds to various pro-

* This work was supported by the Max Planck Society, the Human Frontier Science Program, Deutsche Forschungsgemeinschaft Grants RG562/96 and Sta399/3-1 (to S. S.), and National Institutes of Health Grant R01 NS38051 (to A. A.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF152547.

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[1] The abbreviations used are:, SAF-B, scaffold attachment factor B; EGFP, enhanced green fluorescent protein; GST, gluthatione *S*-transferase; HEK, human embryonic kidney; htra2-β, human transformer-2-beta; hnRNP, heterogenous nuclear ribonucleoprotein; KH domain, hnRNP K homology domain; mAb, monoclonal antibody; Sam68, Src-associated in mitosis; SLM, Sam68-like mammalian protein; SR protein, serine/arginine-rich protein; STAR, signal transduction and activation of RNA.

teins involved in signal transduction such as phospholipase Cγ or p85 phosphatidylinositide 3-kinase, this protein has been proposed to play a role as an adapter protein in fibroblast and lymphocyte signaling (32–35). In addition, there is increasing evidence that Sam68 is important for cell cycle progression (36–38). Recently, two nuclear Sam68-like mammalian proteins (SLM-1 and SLM-2, also called T-STAR/etoile/Salp) have been described (39–41). However, the exact function of Sam68 and its relatives in the nucleus still remains elusive.

Here, we demonstrate for the first time the involvement of the Sam68-like mammalian protein rSLM-2 in the regulation of alternative splicing. This protein interacts with splicing regulatory proteins *in vivo* and *in vitro* and influences the splice site selection of three different minigenes in a concentration-dependent manner. Using a CD44 minigene, we show that the rSLM-2-dependent inclusion of exon v5 depends on a purine-rich sequence to which rSLM-2 binds *in vitro*.

MATERIALS AND METHODS

Two-hybrid Screening and Cloning—A yeast two-hybrid screen and interaction experiments were performed as described (26, 42). Using rSAF-B as a bait in pGBT9, 200,000 colonies of a rat brain embryonic day E16 library (Stratagene, pAdGal4-cDNA as prey) were screened. The DNA of six lacZ-positive clones able to grow on selective medium containing 10 mM 3-aminotriazole was sequenced as described (26). An amino-terminal FLAG tag was introduced to the full-length form or rSLM-2 by polymerase chain reaction followed by subcloning into pcDNA3.1.

Antiserum Production and Purification—Peptides specific for rSLM-2 VVTGKSTLRTRGVTCG and PRARGVPPTGYRPCG were coupled to keyhole limpet hemocyanin and used to immunize rabbits. After 121 days, serum was purified by affinity chromatography, employing a mixture of recombinant GST-rSLM-2 and the two peptides following the manufacturers instructions (Pierce). Dilution for Western blot was 1:1,000 and for immunohistochemistry 1:100. Preabsorption of 10 μl of anti rSLM-2 antibody at a concentration of 0.07 mg/ml with 80 μg rSLM-2 peptides for 30 min at room temperature abolished the specific signal.

Immunoprecipitation and Western Blot—These were performed as described (26). The following antibodies were used: anti-rSLM-2 (1:1,000); anti-SAF-B (1:1,000); anti-p62/SAM68 C20 (Santa Cruz Biotechnology, 1:2,000); anti-htra2-β1 (1:2,000) (25), PY20 (1:10,000), anti-Src (1:1,000) and anti GFP (Boehringer 1:5,000).

In Vitro Protein Interaction Assay—The cDNA of potential interactors of rSLM-2 was cloned into pCR3.1 (Invitrogen) and used for an *in vitro* reticulocyte lysate transcription/translation (TNT, coupled reticulocyte lysate system, Promega) to obtain the corresponding ^{35}S-labeled proteins. For the binding experiments, 2 μl of the reactions were incubated with 1 μg of GST or GST-rSLM-2 coupled to glutathione-Sepharose 4B (Amersham Pharmacia Biotech) in the presence of 200 μl of HNTG buffer and 0.1% Triton X-100 (43) for 2 h at 4 °C. Washing and detection were as described (43).

RNA Gel Shift Assay—RNA gel shift assays were performed as described (24). End labeling of the RNA oligonucleotides was performed with T4 polynucleotide kinase (New England Biolabs) and [γ^{32}P]ATP followed by purification using the nucleotide removal kit (Qiagen). Indicated amounts of recombinant GST-rSLM-2 were incubated with 30 pmol of the ^{32}P-labeled RNA oligonucleotides for 15 min at 30 °C. The oligonucleotide sequences were:

r-rich, GAGGAGGAAAGAGGAGAGAGAAAGGAGGAA;
y-rich, CUCCUCCUUUCUCCUCUCUCUUUCCUCCUU;
v5, AGUAUCAGGAUGAAGAGGAGACCCCACAUGCUACAAGCACAA;
v5ls9, AGUAUCAGGcgacgcgucgGACCCCACAUGCUACAAGCACAA.

In Vivo Splicing Assays—These assays were essentially performed as described (59) employing the CD44v5 (44), the htra2-β1 (23), tau minigenes containing exon 2 or exon 3 (45), or clathrin light chain B minigenes (24, 46). Transfection of the CD44v5 and clathrin light chain B minigenes occurred in HEK293 cells, whereas the htra2-β1 and tau minigenes were analyzed in HN10 cells and COS cells, respectively.

For the CD44v5 and clathrin light chain B minigenes, polymerase chain reaction conditions were as described (24, 44). For the htra2-β minigene, 20-s denaturation at 94 °C, 20-s annealing at 65 °C, and 40-s extension at 72 °C for 33 cycles were used followed by a final extension at 72 °C for 20 min.

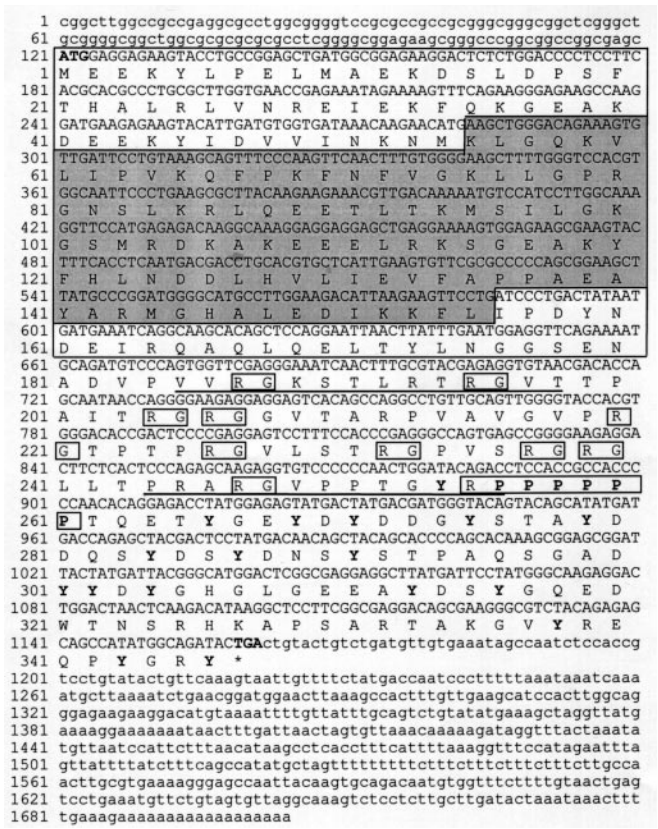

FIG. 1. **cDNA and protein sequence of rSLM-2.** The coding cDNA sequences are indicated in *uppercase*, untranslated regions in *lowercase*. Start and stop codons are shown in *bold*. The protein sequence is shown underneath the cDNA sequence. The KH RNA binding domain is shown as a *shadowed box* and is flanked by the QUA1 and QUA2 regions (indicated as *open boxes*). The arginine and glycine dipeptides clustered in the central part of the protein are *boxed*. The stretch of six prolines and tyrosine residues located in the carboxyl-terminal part of the protein is indicated in *bold*.

For the tau minigenes, polymerase chain reaction was carried out for 22 cycles with 94 °C for 1 min, annealing at 65 °C for 1 min, and extension at 72 °C for 1 min. The resulting splicing pattern was quantified using the Herolab EASY system.

RESULTS

Molecular Cloning and Sequence of rSLM-2—SAF-B was initially cloned because of its strong interaction with scaffold/matrix attachment regions (47). Recently, we described the interaction of SAF-B with RNA polymerase II as well as with splicing factors (7). In addition, SAF-B changed the adenovirus E1A alternative splicing pattern in a concentration-dependent manner (7).

To find new proteins involved in the regulation of alternative pre-mRNA splicing, we used the yeast two-hybrid system to screen an embryonic (E16) rat brain library with rSAF-B (7) as an interacting partner. From about 200,000 colonies screened, six lacZ-positive clones were able to grow in the presence of 5 mM 3-aminotriazole. Two clones contained an open reading frame bearing 67% homology to human Sam68 (27). The predicted protein shares the typical domain structure with members of the STAR family (29) and shows 96% sequence identity to the recently identified mouse proteins etoile/Sam68-like mammalian protein SLM-2 (39–41). It was therefore named rSLM-2. The protein has a maxi-KH homology RNA binding motif (*shadowed box*, Fig. 1). Similar to Sam68, rSLM-2 has a central region containing multiple arginine/glycine (RG) dipeptides, followed by a stretch of six prolines which matches the class I consensus SH3 domain binding site (48). The carboxyl-

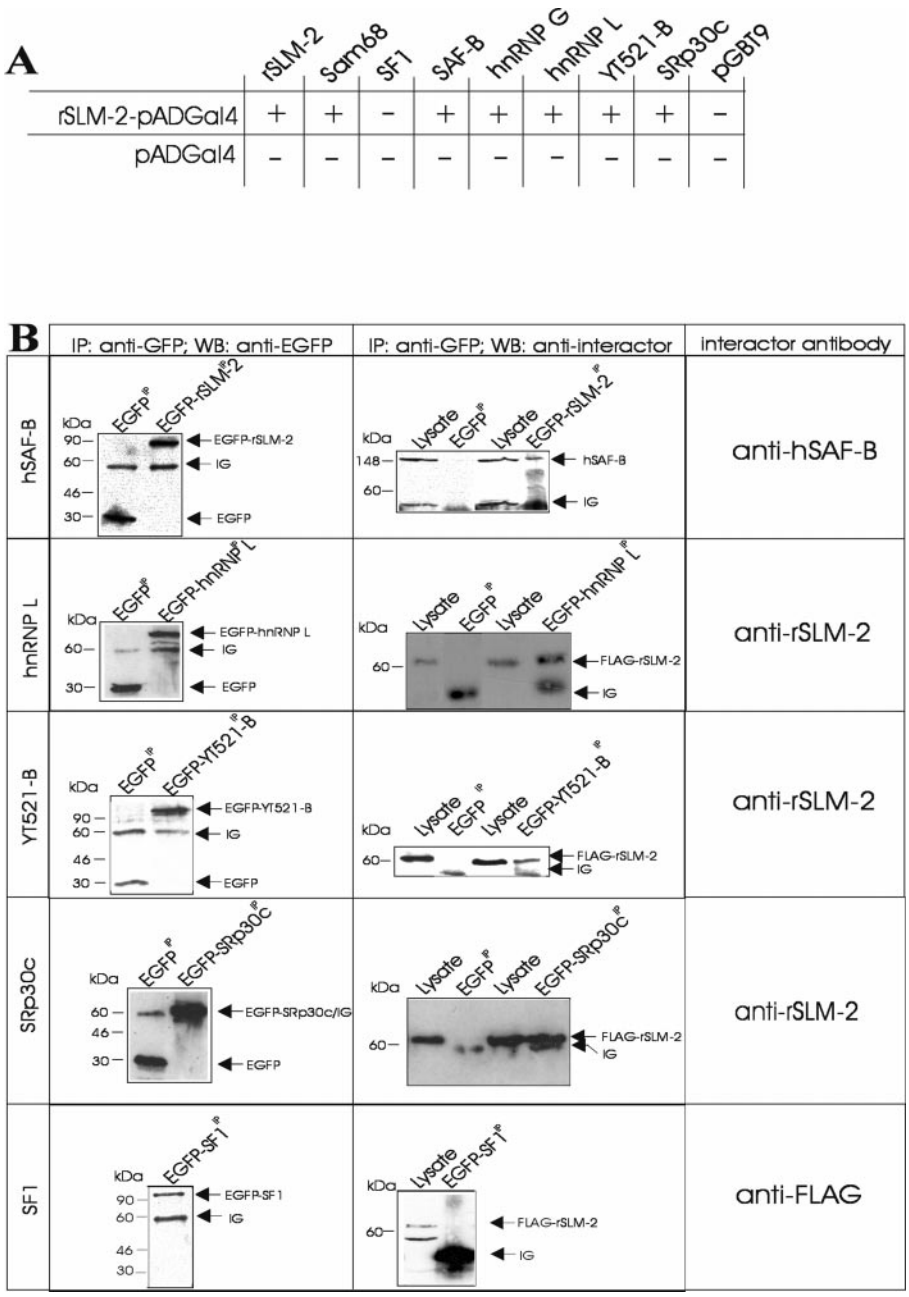

FIG. 2. **Analysis of proteins binding to rSLM-2.** *Panel A*, yeast two-hybrid interactions of rSLM-2. rSLM-2 was fused to the Gal4 activation domain. The rSLM-2 interacting proteins were fused to the Gal4 DNA binding domain (in pGBT9). *Plus signs* indicate growth; *minus signs* indicate no growth on His^- plates containing 5 mM 3-aminotriazole. None of the constructs showed self-activation. *Panel B*, coimmunoprecipitations with rSLM-2. EGFP fusion proteins were overexpressed in HEK293 cells and immunoprecipitated using a monoclonal anti-GFP antibody. The immunoprecipitated protein is indicated by a superscript *IP*. EGFP alone was used as a negative control in each experiment. The immunoprecipitates were analyzed with a polyclonal anti-EGFP antibody (*left column*) to verify successful immunoprecipitation. The immunoprecipitates were also analyzed with interactor-specific antibodies (*middle column*) that are listed in the *right column*. *Arrows* indicate the protein signals observed. *IG*, immunoglobulin signal. Immunoprecipitated rSLM-2 interacts with endogenous SAF-B (*top row*). Immunoprecipitated EGFP-hnRNP L (*second row*), EGFP-YT521-B (*third row*), and EGFP-SRp30c (*fourth row*) bind to FLAG-rSLM-2. Precipitated EGFP-SF1 did not interact with FLAG-rSLM-2 (*fifth row*, *middle column*). The ratio of immunoprecipitation to lysate loaded on the gel was 20:1 in each case.

terminal part of rSLM-2 is rich in tyrosine residues and represents a potential SH2 domain binding site (48, 49).

Identification of Novel Interactors of rSLM-2 Using the Yeast Two-hybrid Screen—To obtain information on the function of rSLM-2, we performed yeast two-hybrid screens with rSLM-2 as an interacting partner in an embryonic rat brain library. It has been shown previously that SLM-2 interacts with the highly homologous protein Sam68 (39), with itself as well as with hnRNP G family members (40). Our two-hybrid results with rSLM-2 confirmed these interactions because we isolated the rat homologs of Sam68 and hnRNP G. In addition, among the lacZ-positive yeast colonies that were able to grow on selective medium containing 5 mM 3-aminotriazol, we identified four novel interactors of rSLM-2: rSAF-B, which has initially been used to clone rSLM-2; the novel splicing-associated protein YT521-B (26, 50); hnRNP L (51); and the SR-protein SRp30c (52) (Fig. 2*A*). In addition, we tested its interaction with another KH domain containing RNA-binding protein, SF1 (53). Although Sam68 was shown to bind to other KH domain-containing proteins such as Bicaudal C or Grp33 (54), rSLM-2 did not interact with the splicing factor SF1, indicating the specificity of the observed interactions.

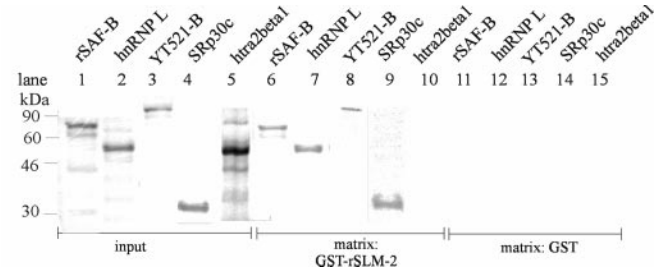

FIG. 3. ***In vitro* interaction assay using recombinant GST-rSLM-2 and radiolabeled hnRNPs or SR proteins.** rSAF-B, hnRNP L, YT521-B, SRp30c, and htra2-β1 were translated *in vitro* in the presence of [^{35}S]Met using a coupled transcription/translation reaction in reticulocyte lysates. These proteins were incubated with GST-rSLM-2 coupled to glutathione-Sepharose 4B. The input of radiolabeled proteins is shown in *lanes 1–5*. Retained proteins were analyzed on a 15% SDS-PAGE (*lanes 6–10*). GST alone was used as a negative control (*lanes 11–15*).

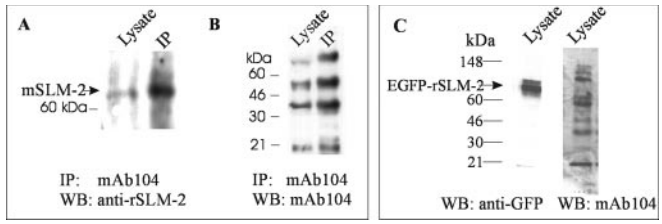

FIG. 4. **Interaction of endogenous rSLM-2 with splicing factors.** *Panel A*, interaction of endogenous rSLM-2 with SR proteins. Immunoprecipitation using the pan SR protein antibody mAb104 (*IP*) was performed with lysates from HN10 cells. rSLM-2 was detected with the rSLM-2 antiserum. The ratio of immunoprecipitate to lysate loaded on the gel was 20:1. *Panel B*, verification of the immunoprecipitation analyzed in *panel A*. One-third of the mAb104 immunoprecipitate was analyzed with the mAB104 antibody (*IP*) and compared with the HN10 lysate. *Panel C*, mAb104 does not cross-react with overexpressed EGFP-rSLM-2. HEK293 cells were transfected with EGFP-rSLM-2. Lysates were probed with the rSLM-2 antiserum (*left*) or mAb104 (*right*), indicating that mAB104 does not cross-react with rSLM-2.

These novel two-hybrid interactions were tested subsequently using coimmunoprecipitations (Fig. 2*B*). We generated a specific antibody against rSLM-2 which did not cross-react with the related proteins Sam68 or rSLM-1.[2] To exclude nucleic acid-mediated interactions, benzonase was present in all experiments. In addition, the RNA-binding protein SF1 (53) was used as a negative control (Fig. 2*B*, *bottom row*). We expressed EGFP-tagged proteins in HEK293 cells and precipitated them using a monoclonal anti-GFP antibody. Overexpressed EGFP was used as a negative control. The efficiency of the immunoprecipitation was analyzed in Western blots with a polyclonal anti-GFP antibody (Fig. 2*B*, *left column*). The coimmunoprecipitating proteins and cell lysates were analyzed by Western blot (Fig. 2*B*, *middle column*) using the appropriate antibody (*right column*). Using this method, we found endogenous hSAF-B to bind to rSLM-2 (Fig. 2*B*, *top row*). In agreement with previous data (39, 40) and our data obtained in yeast, cotransfected hnRNP G and endogenous Sam68 interacted with rSLM-2 (data not shown). Finally, we confirmed the interaction of rSLM-2 with hnRNP L (*second row*), with the splicing associated protein YT521-B (*third row*), and the interaction of rSLM-2 with the splicing factor SRp30c (*fourth row*).

In summary, EGFP-rSLM-2 was able to homomultimerize and to bind to a subset of splicing and splicing-associated factors in the yeast two-hybrid system as well as in coimmunoprecipitations.

rSLM-2 Interacts with hnRNPs and SR Proteins in Vitro—To confirm further the observed interactions of rSLM-2, we performed pull-down experiments using *in vitro* translated rSAF-B, hnRNP L, YT521-B, SRp30c, and tra2-β1 (Fig. 3, *lanes 1–5*), as well as recombinant GST-rSLM-2 protein (Fig. 3, *lanes 6–10*). As shown in *lanes 6–9* in Fig. 3, the hnRNPs rSAF-B and hnRNP L, as well as the splicing-associated protein YT521-B and the SR protein SRp30c, bound directly to rSLM-2. In contrast, htra2-β1 did not bind to GST-rSLM-2 (Fig. 3, *lane 10*), which is in agreement with our data obtained in yeast and demonstrates the specificity of the interactions.

rSLM-2 Forms a Complex with SR Proteins in Vivo—It has been shown previously that the rSLM-2 interactor SAF-B binds to htra2-β1, SRp30c, SF2/ASF, and hnRNP A1 and can change alternative splice sites *in vivo* (7, 55). Therefore, we asked whether rSLM-2 is present in a complex with splicing factors *in vivo*. Screening of several cell lines revealed that SLM-2 is expressed in HN10 cells. These cells are derived from hippocampal neurons (56) that express SLM-2 (Fig. 4*A*, *Lysate*). We performed an immunoprecipitation in HN10 cell lysates with the pan SR antibody mAb104 (57) in the presence of benzonase and found rSLM-2 to be present in this complex (Fig. 4*A*, *IP*). One-third of the corresponding immunoprecipitates were analyzed with the mAb104 antibody (Fig. 4*B*) to verify the successful immunoprecipitation. Furthermore, mAb104 did not cross-react with rSLM-2, even when we overexpressed EGFP-rSLM-2 (Fig. 4*C*). We conclude that endogenous rSLM-2 forms an *in vivo* complex with factors implicated in pre-mRNA splicing.

rSLM-2 Can Change Alternative Splicing Patterns—The association of rSLM-2 with splicing factors as well with the splicing-associated protein YT521-B and hnRNPs points to a role in pre-mRNA processing. Therefore, we asked whether rSLM-2 could modulate splice site selection in different cell lines in a concentration-dependent manner, analogous to several proteins involved in splicing, such as SR proteins (14, 58), hnRNPs (58), SAF-B (7), and YT521-B (26). To investigate this possibility, we employed a CD44 reporter gene, which contains the alternative exon v5 (44). This minigene was transfected with increasing amounts of EGFP-rSLM-2 in HEK293 cells. Vector DNA (pEGFP) was added to ensure that comparable amounts of DNA were transfected in each experiment (59). Increasing the amount of transfected pEGFP-rSLM-2 resulted in an increased incorporation of exon v5, as shown in Fig. 5*A*, suggesting a rSLM-2 concentration-dependent modulation of splice site selection. Western blots with lysates from the transfections confirmed an increase in EGFP-rSLM-2 expression (data not shown).

Next, we determined the role of various rSLM-2 domains and tested deletion variants for their influence on splice site selection. As shown in Fig. 5*B*, deletion of either the RG-rich region and the potential SH2 and SH3 binding domains (rSLM-2Δ2), the tyrosine-rich SH2 domain binding site (rSLM-2Δ3), or the KH domain (rSLM-2Δ4) abolished the influence on splice site selection. All deletion variants were expressed at similar levels in the transfected cells (Fig. 5*C*). We conclude that the ability of rSLM-2 to bind to RNA is necessary for splice site selection. However, in addition to RNA binding, the potential protein interaction sites are also necessary for the splice site switch. This suggests that rSLM-2 acts in a complex with other proteins on pre-mRNA splicing.

We therefore wondered whether proteins binding to rSLM-2 can influence the regulation of alternative splicing of the CD44 minigene and analyzed the interactors SRp30c, hnRNP G, and SAF-B. First, we tested them separately with the CD44 minigene and found that SRp30c slightly repressed exon v5, whereas hnRNP G and SAF-B had no effect on exon v5 usage

[2] O. Stoss, M. Olbrich, A. M. Hartmann, and S. Stamm, manuscript in preparation.

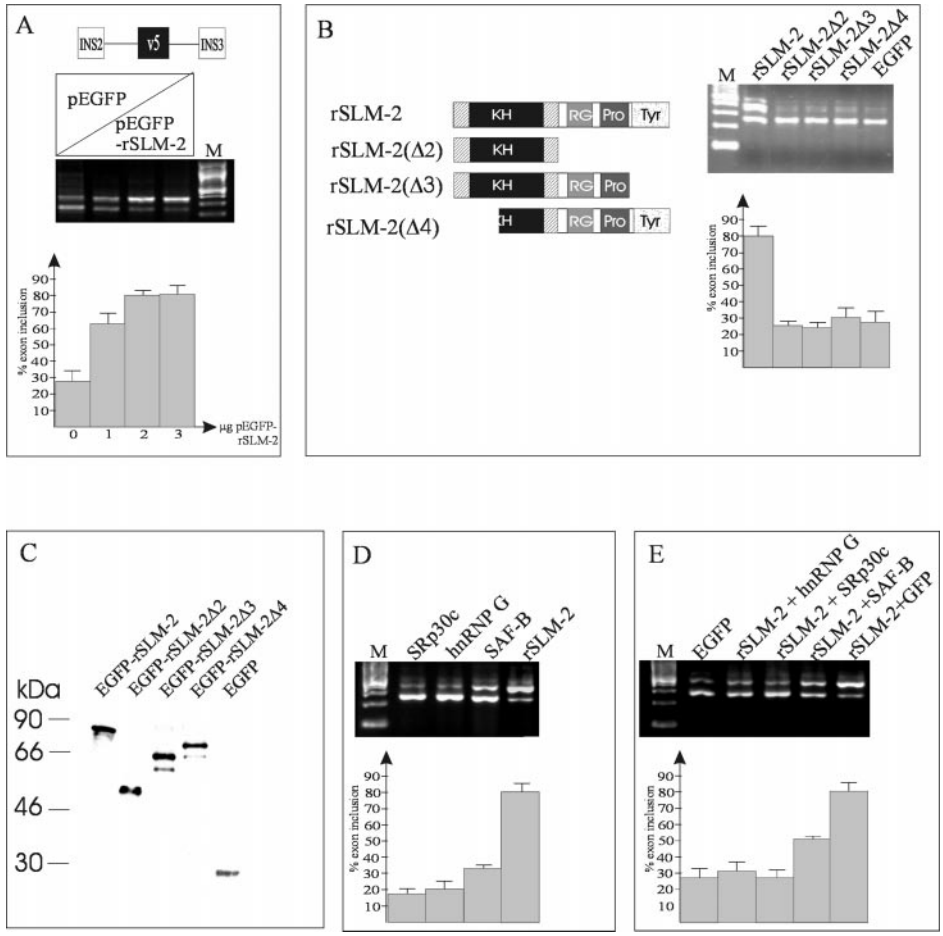

FIG. 5. **rSLM-2 influences alternative splicing patterns in transient transfection assays.** The CD44 minigene containing an alternatively spliced exon was cotransfected with increasing amounts of rSLM-2 followed by reverse transcriptase-polymerase chain reaction analysis of the total RNA using minigene-specific primers flanking the alternative exon. The results of three independent experiments were quantified, and the percentage of exon inclusion is shown below each gel. *M*, 100-base pair ladder. *Panel A*, rSLM-2 promotes the inclusion of the CD44 exon v5. The structure of the minigene is shown schematically on the *top*. INS2 and INS3 are constitutive exons from the preproinsulin gene (44). An increasing amount of pEGFP-rSLM-2 was cotransfected with 2 μg of the CD44v5 minigene. In each titration point, pEGFP was added to keep the total amount of DNA constant. *Panel B*, the CD44v5 minigene was cotransfected with 2 μg of pEGFP, pEGFP-rSLM-2, or its deletion variants (shown on the *left*). *Panel C*, Western blot analysis demonstrating equal amounts of pEGFP-rSLM-2 and its deletion variants in cellular lysates of the transfections. 20 μl of the lysates was analyzed with an anti-GFP antibody. *Panel D*, the CD44v5 minigene was cotransfected with 2 μg of the rSLM-2 interactors SRp30c, htra2-β1, and SAF-B to determine their influence on exon v5 regulation. *Panel E*, the CD44v5 minigene was cotransfected with 2 μg of rSLM-2 in the presence of 2 μg of its interactors.

(Fig. 5*D*). Then, we analyzed these interactors in the presence of rSLM-2 and found that all of them led to a significant decrease of the exon v5 stimulation by rSLM-2 (Fig. 5*E*). These data provide evidence that the rSLM-2-dependent inclusion of exon v5 can be antagonized by SRp30c, hnRNP G, and SAF-B.

The rSLM-2-dependent Exon Inclusion Depends on a Purine-rich Exonic Splicing Enhancer—Pull-down assays on immobilized RNA with HEK293 cell lysates containing overexpressed rSLM-2 showed that rSLM-2 selectively binds to purine-rich RNA (39).[3] Previously, a purine-rich splice enhancer has been characterized in exon v5 of the CD44 gene using linker-scan mutations (44). Because the analysis of rSLM-2 deletion variants demonstrated the necessity of its RNA binding domain in splice site regulation (Fig. 5*B*), we were interested in determining the target sequence of the CD44 pre-mRNA. We compared the influence of rSLM-2 on three different CD44 minigene mutants ls8–ls10 (Fig. 6*A*). In the ls9 variant, the purine-rich enhancer sequence is replaced. Similar to the wild type minigene, EGFP-rSLM-2 was able to induce exon v5 inclusion with the ls8 and ls10 minigene constructs in HEK293 cells (Fig. 6*B*). However, when 10 purine-rich nucleotides have been replaced (ls9), we detected a drastic decrease in the rSLM-2-dependent exon v5 inclusion rate (Fig. 6*A*). Instead of 80% ($\pm$ 4.8) exon v5 inclusion, we only observed 42% ($\pm$ 5.1%) exon inclusion. With EGFP alone, the default splicing pattern was exon v5 skipping in each case.

To determine whether recombinant GST-rSLM-2 binds directly to CD44 exon v5 RNA, we performed gel mobility shift assays with several RNA oligomers. First, we determined that GST-rSLM-2 binds to the purine-rich oligonucleotide r-rich. With increasing amounts of rSLM-2, a super shift is visible which is probably due to the ability of rSLM-2 to multimerize (Fig. 6*C*). This is consistent with our two-hybrid and immunoprecipitation data and the observation that GST-rSLM-2 migrates as a complex of about 450 kDa upon gel filtration (data not shown). Next, we employed the oligonucleotide v5, which contains the regulatory sequence of the CD44 exon v5 and observed binding of rSLM-2 to it (Fig. 6*D*, *lanes 1–3*). Then we used an oligonucleotide (v5ls9) containing the mutation ls9, which abolished the effect of rSLM-2 on CD44 exon v5 regulation *in vivo* (Fig. 6*B*). As shown in Fig. 6*D*, *lanes 4–6*, no binding is observed with the v5Rls9 oligonucleotide. Furthermore, we observed binding to the purine-rich oligonucleotide

[3] O. Stoss, M. Olbrich, A. M. Hartmann, H. König, J. Memmott, A. Andreadis, and S. Stamm, unpublished results.

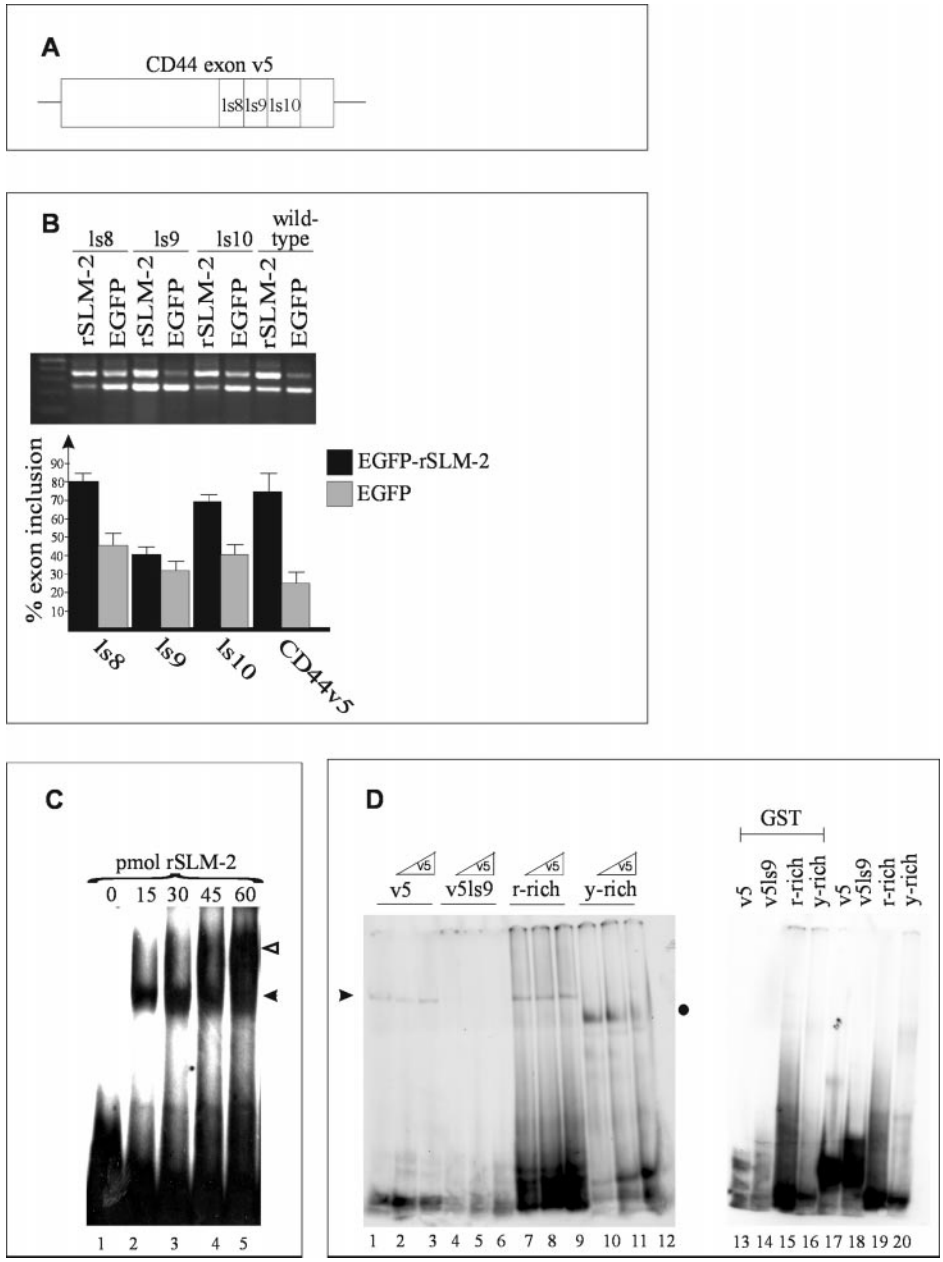

FIG. 6. **rSLM-2-mediated inclusion of exon v5 is dependent on a purine-rich sequence.** *Panel A*, schematic diagram of CD44 exon v5. The locations of the 10-base pair linker scan mutations ls8, ls9, and ls10 within exon v5 are indicated (44). In the mutant ls9 the purine-rich sequence ATGAAGAGGA is replaced by CGACGCGTCG. *Panel B*, the effect of EGFP-rSLM-2 on three different linker scan mutations (ls8–10) in exon v5 of the CD44 minigene has been compared with the effect of EGFP. With the ls8 and ls10 minigenes, 2 μg of transfected EGFP-rSLM-2 increased exon v5 inclusion similar to the wild type minigene. In contrast, only a minor effect of EGFP-rSLM-2 was detected with the ls9 construct. HEK293 cells were used for transient transfections. *Panel C*, recombinant rSLM-2 binds to RNA *in vitro*. An increasing amount of GST-rSLM-2 was incubated with a ^{32}P-labeled polypurine RNA oligonucleotide (r-rich) and separated on a nondenaturing gel. The RNA·rSLM-2 complex is indicated by a *closed arrow*, a larger complex observed at higher rSLM-2 concentration with an *open arrow*. *Panel D*, recombinant rSLM-2 binds the purine-rich exon v5 enhancer *in vitro*. 30 pmol of GST-rSLM-2 was incubated with the RNA oligonucleotides v5, v5ls9, r-rich, and y-rich. Each oligonucleotide was competed with the 20- and 40-fold excess of unlabeled v5 oligonucleotide (*lanes 2* and *3*; *5* and *6*; *8* and *9*; *11* and *12*). Upon longer exposure, binding of the v5ls9 oligonucleotide is observed only in the absence of competitor. The *dot* indicates an unspecific complex seen with the y-rich oligonucleotide that is competed by excess of r-rich oligonucleotide. No shift is observed with GST (*lanes 13–16*) or without protein (*lanes 17–20*).

r-rich (Fig. 6*D*, *lanes 7–9*), but no specific binding to the pyrimidine-rich oligonucleotide y-rich could be detected (Fig. 6*D*, *lanes 10–12*). No binding of these oligonucleotides was observed when GST was used (Fig. 6*D*, *lanes 13–16*). We conclude that rSLM-2 regulates inclusion of CD44 exon v5 by binding to a purine-rich exonic enhancer (see Fig. 8).

Effect of rSLM-2 on Other Minigenes—Next, we tested the effect of rSLM-2 on the alternative splicing of the htra2-β1 gene in HN10 cells (23). Transfection of pEGFP alone leads to 40% inclusion of alternative exon 2. Increasing amounts of rSLM-2 caused a decrease in the β4 isoform and led to an increase in the htra2-β1 isoform (Fig. 7*A*).

Next, we analyzed the effect of rSLM-2 expression on a tau minigene in COS cells. The tau minigene contains the alternatively spliced tau exon 3 (SVΔ2/3) inserted in an insulin expression vector (45). Exons 2 and 3 exhibit the rare incremental combinatorial splicing pattern because exon 3 never appears independently of exon 2. Exons 2 and 3 are adult-specific in the central nervous system (60) but seem to be constitutive in the peripheral nervous system (61, 62). Upon cotransfection, ex-

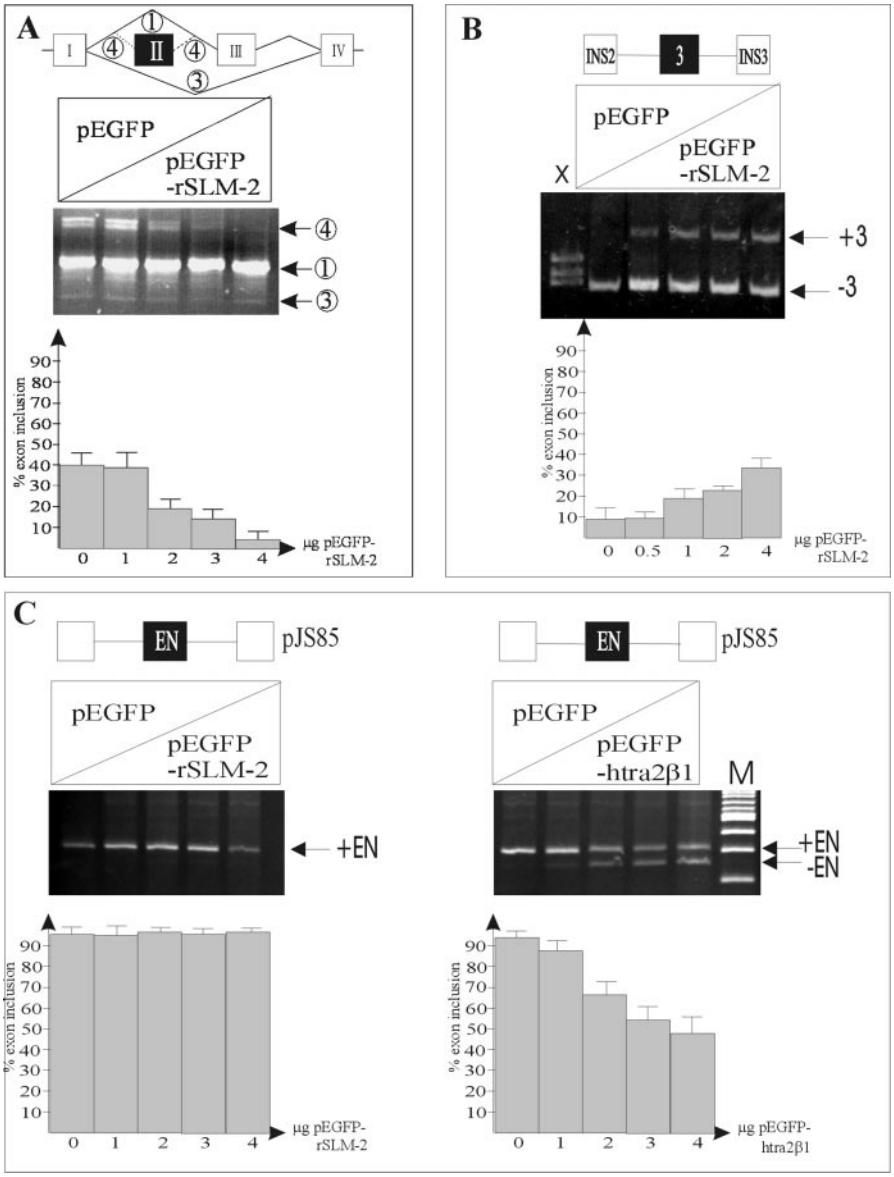

FIG. 7. **The influence of rSLM-2 on the alternative splicing of the htra2-β, tau, and clathrin light chain B pre-mRNA.** The structure of the minigenes is shown on the *top* of each *panel*. A statistical evaluation of three independent experiments is shown below representative ethidium bromide gels. *Error bars* indicate the S.D. *Panel A*, an increasing ratio of pEGFP-rSLM-2 to pEGFP was cotransfected with 2 µg of the htra2-β minigene in HEK293 cells. This increase leads to exon II skipping. *Panel B*, the influence of rSLM-2 concentration on tau minigenes was tested in COS7 cells. 4 µg of the tau minigenes was cotransfected with indicated amounts (µg) of pEGFP-rSLM-2. rSLM-2 leads to an increase in exon 3 inclusion. *X*, phiX *Hae*III digest; *M*, 100-bp ladder. *Panel C*, up to 4 µg of pEGFP-rSLM-2 did not promote exon skipping of clathrin light chain B exon EN in the construct pJS85. In pJS85, exon EN is activated in fibroblasts by an optimized 5′-splice site (24). In contrast, this exon is regulated by htra2-β1 (*right*).

pression of rSLM-2 stimulates exon 3 usage, whereas EGFP alone has no effect (Fig. 7*B*). Interestingly, a sequence stretch in exon 2 of htra2-β and a sequence stretch in exon 3 of the tau transcript show a significant homology to the purine-rich sequence of exon v5 of the CD44 minigene, which is necessary for the rSLM-2-dependent exon inclusion.

Finally, overexpression of rSLM-2 did not stimulate inclusion of the pyrimidine-rich neuron-specific exon EN of the clathrin light chain B transcript (data not shown) and did not repress exon EN usage when this exon is activated by improving its 5′-splice site (Fig. 7*C*). In contrast, htra2-β1 can regulate splice site usage of this construct (Fig. 7*C*), which emphasizes the specificity of rSLM-2-mediated splice site selection. Taken together, this indicates that rSLM-2 can change the alternative splicing pattern of specific substrates in a concentration-dependent manner by binding to purine-rich sequences.

DISCUSSION

rSLM-2 Interacts with Proteins Involved in Splice Site Selection—SAF-B has been shown to associate with the splicing factors SRp30c, ASF/SF2, and htra2-β1 as well as with the carboxyl-terminal domain of the largest subunit of RNA polymerase II, which has also been shown to stimulate pre-mRNA splicing (7, 63). In addition, SAF-B binds to matrix attachment regions and stimulates the generation of the 10S E1A transcript in a concentration-dependent manner (7).

To identify novel proteins involved in mRNA processing, we performed a yeast two-hybrid screen using SAF-B as a bait. This led to the identification of rSLM-2 as a novel interactor of SAF-B.

rSLM-2 and its close relatives Sam68 and rSLM-1 are members of the STAR protein family (29), also called GSG protein family (30). These nuclear proteins combine a slightly modified

CD44 exon v5	**AGG ATG AAG AGG AGA**
htra2beta exon 2	**AGA ATG AAG AAA AGA**
tau exon 2	**AGG CTG CCG CGC AGC**

FIG. 8. **Comparison of purine-rich sequences present in the CD44 exon v5 (44), htra2-β exon2 (23), and tau exon 2 (45).** Identical nucleotides are indicated in *bold*.

hnRNP K homology RNA binding domain, called maxi-KH domain, with binding sites for proteins containing phosphotyrosine or proline binding domains like SH2, SH3, or WW domains. To investigate the function of rSLM-2, we searched for novel interactors using yeast two-hybrid screens and further confirmed these interactions with coimmunoprecipitations and *in vitro* interaction assays using recombinant rSLM-2 protein. Interestingly, all of the rSLM-2-interacting proteins found within these three independent systems are implicated in the regulation of alternative splicing. In addition to SAF-B, which has initially been used to isolate rSLM-2, the splicing-associated protein YT521-B has been found to interact with rSLM-2. Using the SRp20 and htra2-β minigenes, we recently demonstrated the influence of YT521-B on alternative splicing (26). Like rSLM-2, YT521-B interacts with Sam68 and is tyrosine-phosphorylated upon overexpression of the tyosine kinases Src and Fyn (26). It remains to be determined whether rSLM-2 is tyrosine-phosphorylated by nuclear tyrosine kinases, such as SIK/BRK, which was shown to phosphorylate Sam68 (64, 65).

It is notable that rSLM-2, SAF-B, and YT521-B all interact with the splicing factor SRp30c. The physiological significance of this complex formation of rSLM-2 has been demonstrated by endogenous coimmunoprecipitation using the pan anti-SR protein antibody mAb104 (Fig. 4). However, in contrast to SAF-B and YT521-B, no direct binding could be detected between rSLM-2 and the splicing factors htra2-β1 (Figs. 2*A* and 3) or SF1 (Fig. 2). This demonstrates the specificity of the analyzed interactions, which have been carried out in the presence of benzonase to avoid RNA-dependent protein interactions.

Finally, a growing number of hnRNPs have been characterized as molecular players in the regulation of alternative splicing, among them hnRNP A1 (58), hnRNP F, hnRNP H (15), hnRNP I (polypyrimidine tract binding) (16), hnRNP L (66), and a testis-specific member of the hnRNP G family, RNA-binding motif (RBM) (67, 68). Using a two-hybrid screen with RBM as a bait, Venables *et al.* were previously able to isolate the human homologue of rSLM-2, T-STAR (40). Now we provide evidence that rSLM-2 also interacts with the ubiquitously expressed hnRNP G and with hnRNP L.

rSLM-2 Regulates Alternative Splice Site Selection—The binding properties of rSLM-2 indicate a role in pre-mRNA processing. We tested this hypothesis by performing transient transfection assays with several reporter minigenes that contained purine-rich sequences in their alternative exons. As a control, a pyrimidine-rich exon of clathrin light chain B was employed. We found that rSLM-2 changes the splicing patterns of several alternatively spliced exons in a concentration-dependent manner. It induced inclusion of exon v5 of CD44 (44) (Fig. 5*A*), of exon 3 of neurofilament tau (Fig. 7*B*) (69), but caused exon II skipping of the htra2-β exon II (23) (Fig. 7*A*). The opposing effect of rSLM-2 on different minigenes is reminiscent of the effects of splicing factors on natural minigenes *in vivo*. For example, SF2/ASF promotes exon EN inclusion when tested with the clathrin light chain B minigene (58) but causes exon 4 skipping with the SRp20 minigene (70).

Our deletion analysis showed that binding of rSLM-2 to RNA is not sufficient to influence splice site selection. The deletion mutant lacking a functional KH domain rSLM-2(Δ4) is exclusively nuclear (data not shown). In contrast, mutants lacking parts of the carboxyl terminus (rSLM-2Δ2 and Δ3) are also present in the cytosol (data not shown). This indicates that potential protein binding sites such as the tyrosine-rich carboxyl terminus or the proline-rich region are also necessary for activity and proper localization of rSLM-2. Interestingly, the rSLM-2-interacting proteins SAF-B, hnRNP G, and SRp30c inhibited exon v5 inclusion mediated by rSLM-2 (Fig. 5*D*), although these three interactors alone exerted only a minor or no effect on the CD44 minigene. Because SAF-B, hnRNP G, and SRp30c are ubiquitously expressed but rSLM-2 is predominantly expressed in muscle, brain, and testis (40 and data not shown), this suggests that cell type-specific combinations of rSLM-2 and some of its interactors may contribute to different splicing patterns in different cell types. This supports the model that different concentrations of antagonizing factors govern splice site selection.

Binding of rSLM-2 to a purine-rich RNA sequence is necessary to regulate splice site selection because either deleting its RNA binding domain or changing the purine-rich sequence abolishes an effect on usage of exon v5 (Figs. 5*B* and 6*B*).

Because rSLM-2 binds directly to purine-rich RNA (Fig. 6, *C* and *D*), it is likely that it regulates splice site selection through interaction with purine-rich sequences *in vivo*. The *in vitro* binding of rSLM-2 to exon v5 RNA, but not to the mutant v5ls9 (Fig. 6*D*) is in complete agreement with the regulation of CD44 exon v5, but not CD44 exon v5ls9 by rSLM-2 (Fig. 6*B*). In addition, rSLM-2 cannot regulate the pyrimidine-rich exon EN of clathrin light chain B (Fig. 7*C*), even when this exon is activated by a 5′-splice site improvement. A sequence comparison between the regulated exons of CD44, tra2-β1 and tau (Fig. 8) also suggests a purine-rich motif as the likely site of action.

Our data provide for the first time evidence for a role of a STAR protein in the regulation of alternative splicing. In addition, there is increasing evidence that the STAR protein Sam68 is also involved in alternative splice site selection. First, Sam68 has been found to cross-link to an intronic regulatory RNA sequence of the tropomyosin pre-mRNA (71). Second, Bedford *et al.* recently demonstrated the binding of Sam68 to the spliceosome-associated protein FBP21 (72). Finally, like rSLM-2, Sam68 interacts with the testis-specific splicing factor RBM (67). However, a direct effect of Sam68 on alternative splice site selection remains to be shown.

In addition to the role of Sam68 in viral replication (73, 74), two possible functions of rSLM-2 and Sam68 have been investigated to date. First, there is evidence that rSLM-2 and Sam68 are important for cell cycle progression (27, 28, 36, 41), but a direct molecular link is still missing. We speculate that STAR proteins influence the cell cycle by regulating mRNAs necessary for its progression.

Furthermore, Sam68 has been proposed to act as an adapter protein within signal transduction pathways. Upon T cell or insulin receptor stimulation, Sam68 is tyrosine-phosphorylated (33–35). Phosphorylation changes its binding affinities to phospholipase Cγ1, to the regulatory p85 subunit of phosphatidylinositide 3-kinase (32), to itself, and to RNA (54, 75). The p85 phosphatidylinositide 3-kinase also binds to the human homolog of rSLM-2 (41). There are numerous examples for the regulation of alternative splicing by extracellular stimuli (for review, see Ref. 76). Because this list of stimuli which includes insulin (77), nerve growth factor (78), cytokines (79), or neuronal activity (25) is rapidly growing, we are now investigating whether rSLM-2 and similar adapter proteins are part of a signal transduction cascade from receptors toward the spliceosome.

Acknowledgments—We thank Claudia Cap for sequencing and James Chalcroft for artwork.

REFERENCES

1. Singer, R. H., and Green, M. R. (1997) *Cell* **91,** 291–294
2. Misteli, T. (2000) *J. Cell Sci.* **113,** 1841–1849
3. McCracken, S., Fong, N., Yankulov, K., Ballantyne, S., Pan, G., Greenblatt, J., Patterson, S. D., Wickens, M., and Bentley, D. L. (1997) *Nature* **385,** 357–361
4. Kim, E., Du, L., Bregman, D. B., and Warren, S. L. (1996) *J. Cell Biol.* **136,** 19–28
5. Du, L., and Warren, L. (1996) *J. Cell Biol.* **136,** 5–18
6. Cramer, P., Caceres, J. F., Cazalla, D., Kadener, S., Muro, A. F., Baralle, F. E., and Kornblihtt, A. R. (1999) *Mol. Cell* **4,** 251–258
7. Nayler, O., Strätling, W., Bourquin, J.-P., Stagljar, I., Lindemann, L., Jasper, H., Hartmann, A. M., Fackelmayer, F. O., Ullrich, A., and Stamm, S. (1998) *Nucleic Acids Res.* **26,** 3542–3549
8. Neugebauer, K. M., and Roth, M. B. (1997) *Genes Dev.* **11,** 3279–3285
9. Proudfoot, N. (2000) *Trends Biochem. Sci.* **25,** 290–293
10. Cooper, T. A., and Mattox, W. (1997) *Am. J. Hum. Genet.* **61,** 259–266
11. Lui, H.-X., Zhang, M., and Krainer, A. R. (1998) *Genes and Dev.* **12,** 1998–2012
12. Berget, S. M. (1995) *J. Biol. Chem.* **270,** 2411–2414
13. Fu, X.-D. (1995) *RNA* **1,** 663–680
14. Graveley, B. R. (2000) *RNA* **6,** 1197–1211
15. Chou, M. Y., Rooke, N., Turck, C. W., and Black, D. L. (1999) *Mol. Cell Biol.* **19,** 69–77
16. Chan, R. C., and Black, D. L. (1997) *Mol. Cell. Biol.* **17,** 4667–4676
17. Zahler, A. M., Neugebauer, K. M., Lane, W. S., and Roth, M. B. (1993) *Science* **260,** 219–222
18. Hanamura, A., Caceres, J. F., Mayeda, A., Franza, B. R., and Krainer, A. R. (1998) *RNA* **4,** 430–444
19. Kamma, H., Portman, D. S., and Dreyfuss, G. (1995) *Exp. Cell Res.* **221,** 187–196
20. Tian, M., and Maniatis, T. (1993) *Cell* **74,** 105–114
21. King, P. H., Levine, T. D., Fremeau, R. T., and Keene, J. D. (1994) *J. Neurosci.* **14,** 1943–1952
22. Jensen, K. B., Drege, B. K., Stefani, G., Zhong, R., Buchanovich, R. J., Okano, H. J., Yang, Y. Y., and Darnell, R. B. (2000) *Neuron.* **25,** 359–371
23. Nayler, O., Cap, C., and Stamm, S. (1998) *Genomics* **53,** 191–202
24. Stamm, S., Casper, D., Hanson, V., and Helfman, D. M. (1999) *Mol. Brain Res.* **64,** 108–118
25. Daoud, R., Berzaghi, M., Siedler, F., Hübener, M., and Stamm, S. (1999) *Eur. J. Neurosci.* **11,** 788–802
26. Hartmann, A. M., Nayler, O., Schwaiger, F. W., Obermeier, A., and Stamm, S. (1999) *Mol. Biol. Cell* **10,** 3909–3926
27. Taylor, S. J., and Shalloway, D. (1994) *Nature* **368,** 867–871
28. Fumagalli, S., Totty, N., Hsuan, J., and Courtneidge, S. A. (1994) *Nature* **368,** 871–874
29. Vernet, C., and Artzt, K. (1997) *Trends Genet.* **13,** 479–484
30. Chen, T., Boisvert, F. M., Bazett-Jones, D. P., and Richard, S. (1999) *Mol. Biol. Cell* **10,** 3015–3033
31. Jones, A. R., and Schedl, T. (1995) *Genes Dev.* **9,** 1491–1504
32. Richard, S., Yu, D., Blumer, K. J., Hausladen, D., Olszowy, M. W., Connelly, P. A., and Shaw, A. S. (1995) *Mol. Cell. Biol.* **15,** 186–197
33. Sanchez-Margalet, V., and Najib, S. (1999) *FEBS Lett.* **455,** 307–310
34. Lang, V., Mege, D., Semichon, M., Gary-Gouy, H., and Bismuth, G. (1997) *Eur. J. Immunol.* **27,** 3360–3367
35. Fusaki, N., Iwamatsu, A., Iwashima, M., and Fujisawa, J. (1997) *J. Biol. Chem.* **272,** 6214–6219
36. Barlat, I., Maurier, F., Duchesne, M., Guitard, E., Tocque, B., and Schweighoffer, F. (1997) *J. Biol. Chem.* **272,** 3129–3132
37. Resnick, R. J., Taylor, S. J., Lin, Q., and Shalloway, D. (1997) *Oncogene* **15,** 1247–1253
38. Pillay, I., Nakano, H., and Sharma, S. V. (1996) *Cell Growth Differ.* **7,** 1487–1499
39. Di Fruscio, M., Chen, T., and Richard, S. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 2710–2715
40. Venables, J. P., Vernet, C., Chew, S. L., Elliott, D. J., Cowmaedow, R. B., Wu, J., Cooke, H. J., Artzt, K., and Eperon, I. C. (1999) *Hum. Mol. Genet.* **8,** 959–969
41. Lee, J. -S., and Burr, J. G. (1999) *Gene* **240,** 133–147
42. Fields, S., and Song, O. (1989) *Nature* **340,** 245–247
43. Stoss, O., Schwaiger, F. W., Cooper, T. A., and Stamm, S. (1999) *J. Biol. Chem.* **274,** 10951–10962
44. König, H., Ponta, H., and Herrlich, P. (1998) *EMBO J.* **10,** 2904–2913
45. Andreadis, A., Broderick, J. A., and Kosik, K. S. (1995) *Nucleic Acids Res.* **23,** 3585–3593
46. Stamm, S., Casper, D., Dinsmore, J., Kaufmann, C. A., Brosius, J., and Helfman, D. (1992) *Nucl. Acids Res.* **20,** 5097–5103
47. Renz, A., and Fackelmayer, F. O. (1996) *Nucl. Acids Res.* **24,** 843–849
48. Pawson, T. (1995) *Nature* **373,** 573–580
49. Cohen, G. B., Ren, R., and Baltimore, D. (1995) *Cell* **80,** 237–248
50. Nayler, O., Hartmann, A. M., and Stamm, S. (2000) *J. Cell Biol.* **150,** 949–961
51. Pinol-Roma, S., Swanson, M. S., Gall, J. G., and Dreyfuss, G. (1989) *J. Cell Biol.* **109,** 2575–2587
52. Screaton, G. R., Caceres, J. F., Mayeda, A., Bell, M. V., Plebanski, M., Jackson, D. G., Bell, J. I., and Krainer, A. R. (1995) *EMBO J.* **14,** 4336–4349
53. Rain, J.-C., Rafi, Z., Rhani, Z., Legrain, P., and Krämer, A. (1998) *RNA* **4,** 551–565
54. Chen, T., Damaj, B. B., Herrera, C., Lasko, P., and Richard, S. (1997) *Mol. Cell. Biol.* **17,** 5707–5718
55. Weighardt, F., Cobianchi, F., Cartegni, L., Chiodi, I., Villa, A., Riva, S., and Biamonti, G. (1999) *J. Cell Sci.* **112,** 1465–1476
56. Lee, H. J., Hammond, D. N., Large, T. H., Roback, J. D., Sim, J. A., Brown, D. A., Otten, U. H., and Wainer, B. H. (1990) *J. Neurosci.* **10,** 1779–1787
57. Roth, M. B., Murphy, C., and Gall, J. G. (1990) *J. Cell Biol.* **111,** 2217–2223
58. Cáceres, J., Stamm, S., Helfman, D. M., and Krainer, A. R. (1994) *Science* **265,** 1706–1709
59. Stoss, O., Stoilov, P., Hartmann, A. M., Nayler, O., and Stamm, S. (1999) *Brain Res. Prot.* **4,** 383–394
60. Kosik, K. S., Orecchio, L. D., Bakalis, S., and Neve, R. L. (1989) *Neuron* **2,** 1389–1397
61. Goedert, M., Spillantini, M. G., and Crowther, R. A. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 1983–1987
62. Couchie, D., Mavilia, C. I., Georgieff, S., Liem, R. K. H., Shelanski, M. L., and Nunez, J. (1992) *Proc. Natl. Acad. Sci. U. S. A.* **89,** 4378–4381
63. Hirose, Y., Tacke, R., and Manley, J. L. (1999) *Genes and Dev.* **15,** 1234–1239
64. Mitchell, P. J., Barker, K. T., Shipley, J., and Crompton, M. R. (1997) *Oncogene* **15,** 1497–1502
65. Derry, J. J., Richard, S., Valderrama Carvajal, H., Ye, X., Vasioukhin, V., Cochrane, A. W., Chen, T., and Tyner, A. L. (2000) *Mol. Cell. Biol.* **20,** 6114–6126
66. Hahm, B., Cho, O. H., Kim, J. E., Kim, Y. K., Kim, J. H., Oh, Y. L., and Jang, S. K. (1998) *FEBS Lett.* **425,** 401–406
67. Venables, J. P., Elliot, D. J., Makarova, O. V., Makarova, E. M., Cooke, H. J., and Eperon, I. C. (2000) *Hum. Mol. Genet.* **9,** 685–694
68. Elliot, D. J. (2000) *Histol. Histopathol.* **15,** 239–249
69. Wei, M. L., and Andreadis, A. (1998) *J. Neurochem.* **1998,** 1346–1356
70. Jumaa, H., and Nielsen, P. J. (1997) *EMBO J.* **16,** 5077–5085
71. Grossman, J. S., Meyer, M. I., Wang, Y. C., Mulligan, G. J., Kobayashi, R., and Helfman, D. M. (1998) *RNA* **4,** 613–625
72. Bredford, M. T., Frankel, A., Yaffe, M. B., Clarke, S., Leder, P., and Richard, S. (2000) *J. Biol. Chem.* **275,** 16030–16036
73. Reddy, T. R., Xu, W., Mau, J. K., Goodwin, C. D., Suhasini, M., Tang, H., Frimpong, K., Rose, D. W., and Wong-Staal, F. (1999) *Nat. Med.* **5,** 635–642
74. McBride, A. E., Schlegel, A., and Kirkegaard, K. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 2296–2301
75. Wang, L. L., Richard, S., and Shaw, A. S. (1995) *J. Biol. Chem.* **270,** 2010–2013
76. Stoss, O., Stoilov, P., Daoud, R., Hartmann, A. M., Olbrich, M., and Stamm, S. (2000) *Gene Ther. Mol. Biol.* **5,** in press
77. Ishizuka, T., Kajita, K., Yamada, K., Miura, A., Kanoh, Y., Ishizawa, M., Wada, H., Itaya, S., Yamamoto, M., Yasuda, K., Nagata, K., and Okano, Y. (1996) *Diabetes Res. Clin. Pract.* **33,** 159–167
78. Smith, M. A., Fanger, G. R., O'Connor, L. T., Bridle, P., and Maue, R. A. (1997) *J. Biol. Chem.* **272,** 15675–15681
79. McKay, N. G., Hunter, D. J., Haites, N. E., and Power, D. A. (1994) *Biochem. Biophys. Res. Commun.* **199,** 1005–1011