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General splicing factors SF2 and SC35 have equivalent activities *in vitro*, and both affect alternative 5′ and 3′ splice site selection

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ABSTRACT **The human pre-mRNA splicing factors SF2 and SC35 have similar electrophoretic mobilities, and both of them contain an N-terminal ribonucleoprotein (RNP)-type RNA-recognition motif and a C-terminal arginine/serine-rich domain. However, the two proteins are encoded by different genes and display only 31% amino acid sequence identity. Here we report a systematic comparison of the splicing activities of recombinant SF2 and SC35. We find that either protein can reconstitute the splicing activity of S100 extracts and of SC35-immunodepleted nuclear extracts. Previous studies revealed that SF2 influences alternative 5′ splice site selection *in vitro*, by favoring proximal over distal 5′ splice sites, and that the A1 protein of heterogeneous nuclear RNP counteracts this effect. We now show that SC35 has a similar effect on competing 5′ splice sites and is also antagonized by A1 protein. In addition, we report that both SF2 and SC35 also favor the proximal site in a pre-mRNA containing duplicated 3′ splice sites, but this effect is not modulated by A1. We conclude that SF2 and SC35 are distinct splicing factors, but they display indistinguishable splicing activities *in vitro*.**

Nuclear pre-mRNA splicing takes place in spliceosomes, high molecular weight complexes containing small nuclear ribonucleoprotein (snRNP) particles and non-snRNP protein splicing factors (1). Our laboratories have identified and characterized two distinct non-snRNP splicing factors designated SF2 (splicing factor 2) (2–4) and SC35 (spliceosome component of 35 kDa) (5, 6). SF2 is also known as ASF or ASF-1 (alternative splicing factor) (7, 8), whereas SC35 has also been termed PR264 (9). In separate studies, SF2/ASF (3, 7) and SC35 (5, 10, 11) were shown to be required for the first step of splicing and for the assembly of the earliest detectable ATP-dependent prespliceosome complex (A complex). SF2 was originally identified by its ability to complement splicing-deficient HeLa cell S100 extracts (2), and SC35 by its ability to complement nuclear extracts immunodepleted with an anti-SC35 monoclonal antibody (mAb) raised against partially purified mammalian spliceosomes (5, 10). In addition, SF2 was shown to activate preferentially the proximal 5′ splice site in pre-mRNAs containing two or more alternative 5′ splice sites (4, 7, 12), a reaction that is specifically counteracted by the A1 protein of heterogeneous nuclear RNP (hnRNP) particles (13). A systematic comparison of the activities of SF2 and SC35 was previously not possible, since homogeneous preparations of each protein were not available.

SF2 and SC35 are phosphoproteins that migrate in SDS/polyacrylamide gels as doublets, with very similar apparent molecular masses (33–35 kDa), and the two proteins copurify through multiple chromatographic steps (3, 6, 10). These observations, in conjunction with the requirement of both factors for complex A assembly, suggested that they may actually correspond to the same protein. The slight differences in apparent molecular mass could be due to differences in posttranslational modifications. However, comparison of the nucleotide sequences of SF2/ASF-1 (8, 14) and SC35/PR264 (6, 9) cDNA clones revealed that they encode highly related but distinct polypeptides. SF2 is a 248-amino acid (aa) protein of 27,744 Da, whereas SC35 is a 221-aa protein of 25,575 Da, not taking into account posttranslational modifications. Both SF2 and SC35 contain a RNP-type RNA-recognition motif (RRM) (15, 16) and C-terminal arginine and serine repeats (RS domain), but they display only 31% sequence identity. The two cDNAs encode proteins with splicing activities indistinguishable from those purified from HeLa cell nuclear extracts. Recombinant SF2 produced in bacteria complemented S100 extracts and influenced 5′ splice site selection *in vitro* (8, 14), whereas recombinant SC35 produced in a baculovirus system could complement anti-SC35 immunodepleted extracts (6).

Recently, both proteins were shown to be members of the SR family of phosphoproteins (17–19). Proteins in this family are recognized by mAb 104, which is specific for a shared phospho-epitope; copurify in a highly selective two-step purification procedure; and contain an N-terminal RRM and a C-terminal RS domain (19). The SR family consists of at least five members of apparent molecular mass 20, 30, 40, 55, and 75 kDa, which are highly conserved between *Drosophila* and man. The 55-kDa polypeptide, SRp55 (17), isolated from a *Drosophila* cell line, can complement HeLa cell S100 extracts for splicing and causes the preferential selection of proximal 5′ splice sites (18). Moreover, each of the 30-, 40-, 55-, and 70-kDa SR polypeptides from calf thymus, recovered individually from SDS/polyacrylamide gels, can complement HeLa cell S100 extracts for splicing (19). Partial amino acid sequence analysis of the human 30-kDa species revealed both SF2 and SC35 polypeptides, respectively designated SRp30a and SRp30b (19). Thus, it was not clear whether the *in vitro* splicing activity of the purified 30-kDa protein was due to the presence of SF2, SC35, or both.

Here we compare the splicing activities of recombinant SF2 and SC35. We find that the two factors have indistinguishable activities *in vitro*. Both factors can complement S100 extracts for splicing, and SF2 can substitute for SC35 in complementing extracts immunodepleted with the anti-SC35 mAb. In addition, both SF2 and SC35 favor proximal over distal 5′ splice sites, and this proximal splice site selection activity of both proteins is counteracted by hnRNP A1. Finally, both SF2 and SC35 can also influence 3′ splice site selection in a pre-mRNA containing competing 3′ splice sites. This latter activity is not affected by hnRNP A1.

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Abbreviations: RNP, ribonucleoprotein; hnRNP, heterogeneous nuclear RNP; snRNP, small nuclear RNP; mAb, monoclonal antibody; RRM, RNA-recognition motif; aa, amino acid(s).

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MATERIALS AND METHODS

Pre-mRNA Substrates. Wild-type β-globin plasmid template (HβΔ6) and derivatives containing a 5′ splice site duplication (5′D16X) or a 3′ splice site duplication (3′D115) were described previously (20, 21). Capped, ^{32}P-labeled RNAs were prepared from *Bam*HI-linearized templates by *in vitro* transcription with SP6 RNA polymerase (2).

Recombinant Proteins. SF2 was expressed in *Escherichia coli* and purified (14). SC35 was expressed in Sf9 cells in a baculovirus vector and purified (6). The hnRNP A1 protein was expressed in *E. coli* and purified (13). The concentrations of purified recombinant proteins used were approximately 0.13 mg/ml (SC35), 0.06 mg/ml (SF2), and 0.2 mg/ml (hnRNP A1). Some experiments employed purified HeLa cell SF2 (0.13 mg/ml) that was shown to be devoid of SC35 (A.M. and A.R.K., unpublished data).

Splicing Reactions. HeLa cell nuclear and S100 extracts were prepared as described (2). SC35-depleted nuclear extract was prepared by using anti-SC35 mAb bound to protein G-agarose (6). Standard 25-μl reaction mixtures (20, 22) contained the amounts of nuclear extract, S100 extract, and recombinant proteins indicated in each figure legend. All extracts and fractions were dialyzed in modified buffer D (2). All reactions were supplemented with the same buffer, when necessary, to bring the total amount of buffer to 15 μl.

RESULTS

SF2 and SC35 Complement the Same Splicing-Deficient Extracts. Recombinant SF2 and human or bovine 30-kDa SR protein (SRp30) isolated from preparative SDS/polyacrylamide gels can complement S100 extracts for splicing (4, 18, 19). However, gel-purified SRp30 contains both SF2 and SC35 (19). To determine whether SC35 alone can complement the S100 extract, we compared the splicing activities of recombinant SF2 and SC35. Comparable levels of β-globin pre-mRNA splicing were observed when equivalent amounts of SF2 and SC35 were used to complement HeLa cell S100 extract (Fig. 1*A*, lanes 2 and 4; Fig. 1*B*, lanes 3 and 4). Thus, the S100 extract is deficient in both SF2 and SC35, and either protein can reconstitute splicing activity.

We next tested whether recombinant SF2 could complement SC35-depleted HeLa cell nuclear extract for splicing. SF2 efficiently complemented the SC35-depleted nuclear extract (Fig. 2). This particular preparation of SF2 was less active than SC35 in both complementation assays (lanes 4 and 6), but this is unlikely to reflect a biologically significant difference, since *E. coli*-expressed SF2 was subjected to a denaturation–renaturation protocol and is sensitive to repeated freezing and thawing. Since SF2 and SC35 are functionally redundant *in vitro*, and SC35-depleted extracts are not active, it appears that SF2 and SC35 may be codepleted by the anti-SC35 mAb. Indeed, immunoblot analysis of anti-SC35- and control mAb-immunodepleted nuclear extracts indicated that anti-SC35 mAb immunodepletion resulted in the partial but selective depletion of not only SC35 but also SF2 and SRp75 (data not shown). Coimmunodepletion appears to be due, at least in part, to crossreactivity of the anti-SC35 mAb with SF2, which can be observed by immunoblotting of recombinant SF2 expressed in baculovirus, but not of SF2 expressed in bacteria (data not shown), consistent with the finding that this mAb recognizes a phospho-epitope (X.-D.F. and T.M., unpublished data). In addition, coimmunodepletion may reflect the existence of the SR proteins in a common high molecular weight complex in nuclear extracts (see *Discussion*).

SF2 and SC35 Stimulate Proximal 5′ Splice Sites *in Vitro*. Human SF2 and *Drosophila* SRp55 can modulate 5′ splice site selection *in vitro* with alternatively spliced pre-mRNAs,

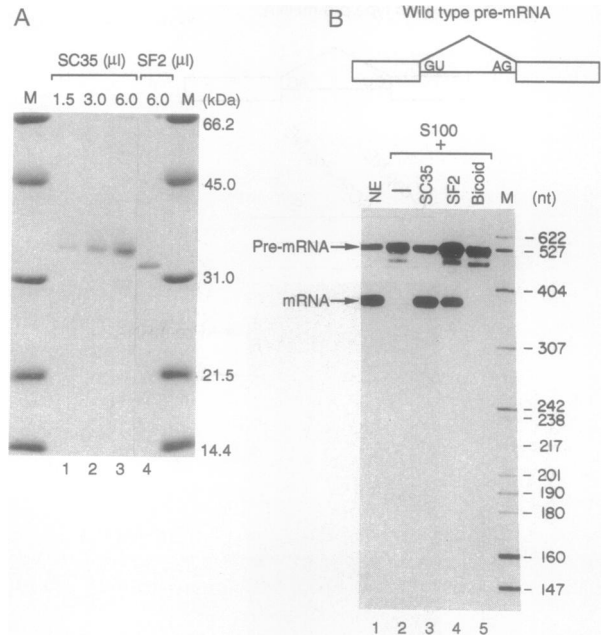

FIG. 1. Recombinant SF2 and SC35 complement HeLa cell S100 extracts. (*A*) Purified recombinant SF2 and SC35. SF2 was produced in *E. coli* and SC35 was produced by baculovirus. The indicated amounts of SF2 and SC35 were analyzed by SDS/PAGE and Coomassie blue R250 staining. The slower migration of SC35 is caused by phosphorylation in the Sf9 insect cells. Lanes M, size markers. (*B*) Splicing activity of recombinant SF2 and SC35. Human β-globin pre-mRNA was incubated under splicing conditions in HeLa nuclear extract (NE, 4 μl; lane 1) or in S100 extract alone (7 μl) or supplemented with 3 μl of recombinant SC35 (lane 3), 6 μl of recombinant SF2 (lane 4), or 6 μl of recombinant bicoid protein as a negative control (lane 5). Positions of pre-mRNA and mRNA are indicated. Lane M, size markers. nt, Nucleotides.

so that proximal 5′ splice sites are favored at high SF2 or SRp55 concentrations (4, 7, 8, 12, 14, 18). To investigate whether recombinant SC35 has a similar effect on 5′ splice site selection *in vitro*, we analyzed the splicing pattern of a model pre-mRNA containing a duplicated 5′ splice site (5′D16X) (21) in nuclear extract and in S100 extract supplemented with recombinant SC35. In nuclear extract the distal 5′ splice site of 5′D16X pre-mRNA was preferentially used (Fig. 3, lane 1). However, with increasing amounts of SC35 the proximal 5′ splice site was selected with increasing efficiency, while use of the distal 5′ splice site remained approximately the same (lanes 2–4). When the 5′D16X pre-mRNA was spliced in S100 extract plus SC35, selection of the distal 5′ splice site became very inefficient (lanes 6 and 7), and a complete switch to the proximal 5′ splice site was obtained as the concentration of SC35 increased (lane 8). An essentially identical pattern of 5′ splice site selection was previously reported with this substrate and purified HeLa cell or recombinant SF2 (refs. 4 and 14; see also lane 9). As expected, no splicing activity or 5′ splice site switching was observed when recombinant bicoid protein was added (lane 10 and data not shown). We conclude that SC35 and SF2 are indistinguishable in the 5′ splice site selection assay.

The 5′ Splice Site Selection Activities of SF2 and SC35 Are Counteracted by hnRNP A1. The 5′ splice site selection patterns of several alternatively spliced pre-mRNAs are different in nuclear extracts, or in SF2-supplemented nuclear extracts, from those observed in S100 extracts complemented with SF2 (refs. 4 and 13; see also Fig. 3). Recently, this difference was shown to be due to the high concentration of hnRNP A1 in nuclear extract, which counteracts the SF2 effect on alternative 5′ splice site selection *in vitro* and thereby preferentially stimulates distal 5′ splice sites (13). A

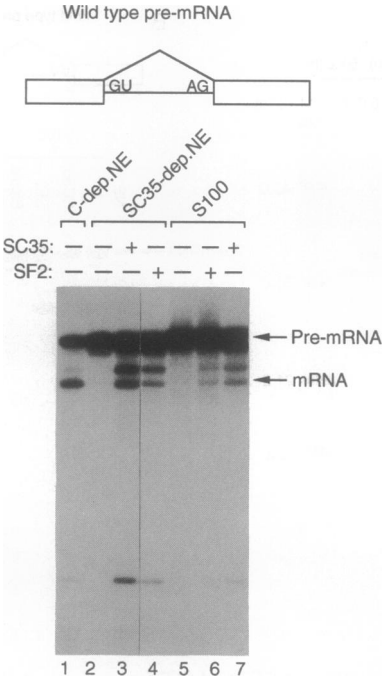

FIG. 2. Recombinant SF2 and SC35 complement HeLa nuclear extract immunodepleted with the anti-SC35 mAb. Nuclear extract (NE) was depleted with either an isotype-matched control mAb (lane 1) or anti-SC35 mAb (lanes 2–4). The SC35-depleted nuclear extract (7.5 μl) was complemented with 3 μl of SC35 (lane 3) or with 6 μl of SF2 (lane 4). Complementation of the S100 extract (7 μl) with SF2 (6 μl; lane 6) and SC35 (3 μl; lane 7) was included for comparison of their relative activities in these two complementation systems. Substrate was wild-type human β-globin pre-mRNA.

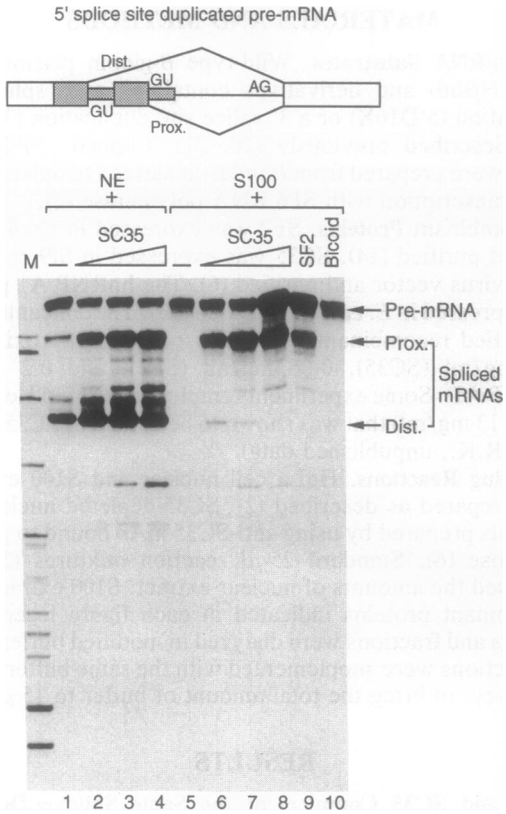

FIG. 3. Recombinant SC35 modulates 5′ splice site selection *in vitro*. Recombinant SC35 was added in increasing amounts to 4 μl of nuclear extract (NE, lanes 1–4) or to 7 μl of S100 extract (lanes 5–8) programmed with the 5′D16X pre-mRNA, which contains duplicated 5′ splice sites. Lanes 1 and 5, no addition; lanes 2 and 6, 1.5 μl; lanes 3 and 7, 3 μl; lanes 4 and 8, 6 μl. S100 was also complemented with 6 μl of SF2 (lane 9) or with 6 μl of bicoid (lane 10) as positive and negative controls, respectively. Positions of the mRNA products generated by selection of proximal (Prox.) or distal (Dist.) 5′ splice sites are indicated. Lane M, size markers as in Fig. 1*B*.

similar activity, termed DSF, was partially purified from HeLa cells (23), but it is not known if it is identical to hnRNP A1. To determine whether hnRNP A1 can oppose SC35-mediated 5′ splice site selection *in vitro*, we carried out similar experiments. We found that increasing amounts of hnRNP A1 in S100 extracts complemented with a fixed amount of SC35 resulted in a switch from the proximal to the distal 5′ splice site (Fig. 4, lanes 2–7). When a fixed amount of hnRNP A1 was added to the S100 extract, increasing amounts of SC35 had an opposite effect, resulting in an increase in the use of the proximal 5′ splice site (lanes 8–12). Thus, hnRNP A1 has similar antagonistic effects on SC35 and SF2 in 5′ splice site selection *in vitro*.

SF2 and SC35 Influence 3′ Splice Site Selection *in Vitro*. SF2, SC35, and SRp55 can strongly influence 5′ splice site selection *in vitro* (see above), whereas only a limited effect on 3′ splice site selection by SF2 was previously reported (4). Since SC35 appears to be one of the splicing factors that mediate U1 and U2 interactions at the 3′ splice site (11), it is possible that SC35 and other SR proteins can also affect 3′ splice site choice *in vitro*. To test this possibility, we used a pre-mRNA containing duplicated 3′ splice sites (3′D115, ref. 21; Fig. 5) and examined its splicing patterns in dilute nuclear extract in the absence, or in the presence of variable amounts, of SC35 or SF2. SC35 had a selective, dose-dependent effect on 3′ splice site selection of the 3′D115 pre-mRNA by promoting the use of its proximal site (Fig. 5, lanes 1–3). A virtually identical concentration-dependent effect on 3′ splice site selection was mediated by SF2 (lanes 4 and 5). We conclude that both SC35 and SF2 influence 3′ as well as 5′ splice site selection *in vitro*. In contrast to the 5′ splice site switching activity, the preferential activation of the proximal 3′ splice site of 3′D115 by SF2 and SC35 was not counteracted by hnRNP A1 (data not shown).

DISCUSSION

We report the results of a systematic comparison of the *in vitro* splicing activities of recombinant SF2 and SC35. These two splicing factors were initially identified by very different approaches but appeared to be remarkably similar in their physical properties, biochemical fractionation, and requirement for spliceosome assembly. However, the two activities were later shown to represent homologous (31% amino acid sequence identity) but distinct polypeptides. Our analysis of the recombinant proteins did not reveal any differences in their activities *in vitro*. Both proteins efficiently complement HeLa cell S100 extracts, as well as nuclear extracts immunodepleted with anti-SC35, and influence 5′ and 3′ splice site selection in pre-mRNAs containing competing splice sites. Thus, the *in vivo* function of the two proteins remains unknown. The two proteins could be entirely redundant in their activities *in vivo*, they could preferentially associate with particular pre-mRNAs or splice sites, or they could be expressed at different levels in different cell types.

Other members of the SR family of splicing factors can complement S100 extracts (18, 19), and *Drosophila* SRp55 can influence 5′ splice site selection in a human *in vitro* system (18). The activities of the remaining SR proteins in alternative 5′ or 3′ splice site selection *in vitro* have not been reported. Although it is possible that a careful analysis of the *in vitro* activities of recombinant SR proteins may reveal some differences, functional differences may be discernible only *in vivo*. The conservation of the entire set of SR proteins

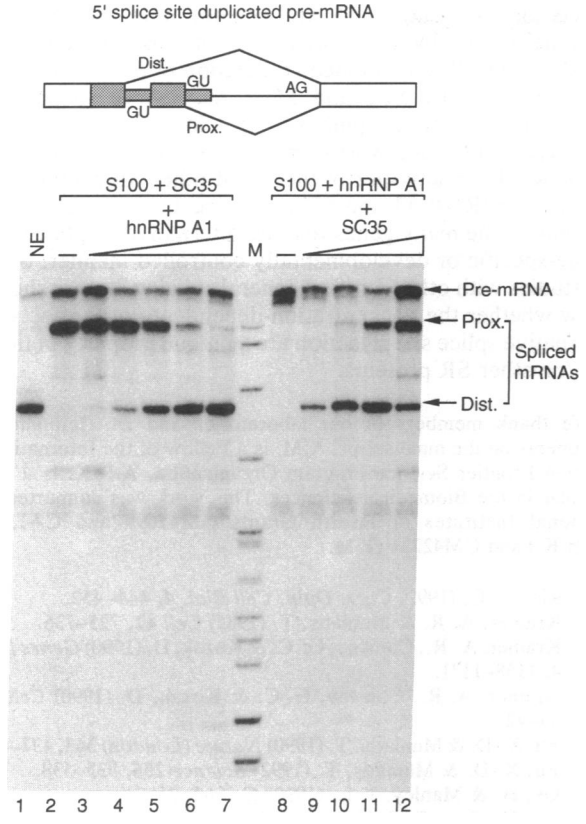

FIG. 4. hnRNP A1 counteracts the effect of SC35 on 5′ splice site selection. S100 extract (6 μl) was complemented with 3 μl of recombinant SC35 (lanes 2–7) and increasing amounts of recombinant hnRNP A1 (lane 2, no addition; lane 3, 0.5 μl; lane 4, 1 μl; lane 5, 2 μl; lane 6, 4 μl; lane 7, 6 μl) or with 3 μl of hnRNP A1 (lanes 8–12) and increasing amounts of SC35 (lane 8, no addition; lane 9, 0.75 μl; lane 10, 1.5 μl; lane 11, 3 μl; lane 12, 6 μl). Nuclear extract (lane 1, 8 μl) was included as a control. Substrate was 5′D16X pre-mRNA. Positions of the mRNA products generated by selection of proximal or distal 5′ splice sites are indicated. Lane M, size markers (Fig. 1*B*).

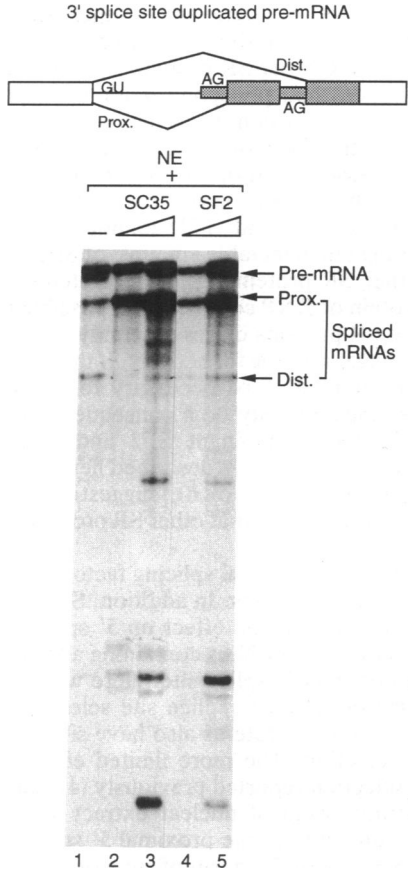

FIG. 5. SF2 and SC35 modulate 3′ splice site selection *in vitro*. Splicing of the 3′D115 pre-mRNA, which contains duplicated 3′ splice sites, was carried out in nuclear extract (NE, 3 μl) supplemented with recombinant SC35 (lane 1, no addition; lane 2, 2 μl; lane 3, 8 μl) or purified HeLa cell SF2 (lane 4, 2 μl; lane 5, 8 μl). Positions of the mRNA products generated by selection of proximal or distal 3′ splice sites are indicated.

between *Drosophila* and man strongly suggests that the proteins are not entirely redundant *in vivo*. Thus, although the functions that are currently measured *in vitro* tolerate substantial size and sequence divergence—e.g., between human SF2 (248 aa) and human SC35 (221 aa) (31% identity)—distinct members of the family have been remarkably conserved phylogenetically. For example, human SF2 (SRp30a) and *Drosophila* SRp55 (350 aa) exhibit 50% identity (18) even though they are not true homologues: partial amino acid sequence of purified proteins showed that there is a homologue of SRp55 in vertebrates and of SRp30a in *Drosophila*, each conserved in size and sequence (19). Homologous members of the family exhibit even greater conservation: human and avian SC35/PR264 (SRp30b; 98% identity) (9), mouse X16 and human SRp20 (164 aa; 100% identity) (19, 24), human SRp20 and *Drosophila* rbp1 (135 aa, Y.-J. Kim and B. S. Baker, personal communication; 63% identity). The possibility that at least some members of the SR family may function in a tissue-specific or developmentally regulated manner is suggested by the observation that expression of mouse X16 (SRp20) mRNA is high in thymus, spleen, and testes but low or undetectable in liver, kidney, brain, and heart (24). Similarly, variable levels of SC35/PR264 mRNAs are expressed in different cell lines and stages of development (6, 9), and the abundance of SF2 protein varies considerably in different adult rat tissues (A.R.K., unpublished data).

A number of observations suggest that SF2, SC35, and other SR proteins may be associated with each other in the cell. (*i*) SR proteins colocalize in discrete nuclear structures along with other spliceosome components such as snRNPs (25). This was demonstrated by immunofluorescence staining, confocal laser scanning, and/or immuno-electron microscopy with the mAbs anti-SC35 (5, 10), anti-SF2 (D. Spector and A.R.K., unpublished data), and 104, which recognizes all SR family members (26). (*ii*) HeLa cell S100 extracts appear to be deficient in all SR family proteins, but contain all other essential spliceosomal components. Thus, as discussed above, SF2, SC35, and all other SR family proteins thus far tested can reconstitute the splicing activity of S100 extracts. The spliceosomal components present in these "cytoplasmic" S100 extracts leak out of the nucleus when the cells are lysed in a hypotonic buffer (2). It is therefore possible that the absence of SR proteins from S100 extracts is due to association of these proteins to form a protein or RNP complex too large to leak out of the nucleus at low salt concentrations (3). Alternatively, the SR proteins may be pelleted during the standard ultracentrifugation of the S100 extract in the presence of Mg^{2+} (19). We favor the former explanation because omission or chelation of Mg^{2+} during the ultracentrifugation step does not yield an S100 extract that is active in splicing in the absence of added SR proteins, and because a similar spin in the presence of Mg^{2+} does not inactivate a nuclear extract (unpublished data). (*iii*) SF2 activity is present in rapidly sedimenting endogenous RNP complexes in nuclear extracts and can be released from these complexes by incubation in the presence of ATP (27). (*iv*) Nuclear extracts that are immunodepleted with the

anti-SC35 mAb are also deficient in other SR family members. The anti-SC35 mAb shows variable crossreactivity with denatured SF2 and other SR proteins by immunoblot analysis, depending on their phosphorylation states, demonstrating the existence of a conserved epitope. However, codepletion of the native SR proteins with the anti-SC35 mAb may be due to the association of all SR proteins to form a high molecular weight complex in nuclear extracts.

Both S100 extracts and SC35-immunodepleted extracts contain limiting but detectable amounts of SF2 and SC35, and probably other SR proteins. If these proteins are part of a common protein or RNP complex, it is possible that addition of any of the SR proteins drives assembly of the complex so that it is no longer limiting for activity. If this is the case, each of the SR proteins may be necessary for activity, and the apparent redundancy may be a consequence of incomplete depletion. When recombinant SF2 and SC35 are added together in any of the assays described here, their effects are only additive (data not shown), suggesting that these two proteins are redundant or that other SR proteins have become limiting.

SF2 and SC35 are general splicing factors and are nonspecific RNA-binding proteins. In addition, SF2 was previously shown to have a dramatic effect on 5′ splice site selection with a variety of pre-mRNAs containing alternative 5′ splice sites and a common 3′ splice site. Here we show that SC35 has the same effect on 5′ splice site selection as SF2 and, further, that the two proteins also have similar effects on 3′ splice site selection. The more limited effect of SF2 on 3′ splice site selection reported previously (4) can be attributed to a suboptimal ratio of nuclear extract and SF2. Just as hnRNP A1 antagonizes the proximal 5′ splice site selection activity of SF2 and SC35, a pair of antagonistic activities, SF6 and SF7, determine the selection of competing 3′ splice sites *in vitro* (A.M. and A.R.K., unpublished work). Whereas SF6 activity, which favors proximal 3′ splice sites, is present in SF2 and SC35, an excess of SF7 causes the selection of distal 3′ splice sites. To minimize SF7 activity, less nuclear extract was employed in the present study. An activity that stimulates use of a proximal duplicated 3′ splice site in a δ-crystallin model pre-mRNA has been partially purified (N. Kataoka and Y. Shimura, personal communication) and may prove to be one of the SR proteins.

The hnRNP A1 protein counteracts the effects of both SF2 and SC35 on 5′ splice site selection *in vitro*, but the mechanism of this effect is not understood. The effect is specific, since hnRNP A1 does not inhibit general splicing, which requires SR proteins, and actually slightly enhances splicing (13). Moreover, no analogous effects of hnRNP A1 at 3′ splice sites were observed with the substrates tested so far (ref. 13; this study). One possibility is that hnRNP A1 and splicing factors compete for access to the 5′ splice sites (13). The equilibrium binding of hnRNP A1 to single-stranded polynucleotides is cooperative and shows limited base specificity (28). *In vitro* reconstitution studies with hnRNP core proteins also show a lack of sequence specificity (29). However, the contacts of hnRNP A1 with pre-mRNA are sensitive to the presence of additional factors in nuclear extracts (30), and specific binding of hnRNP A1 to the 5′ (D. Stolow and S. Berget, personal communication) and 3′ (31) splice sites under splicing conditions has been reported. The differences in the effects of hnRNP A1 on 5′ and 3′ splice site selection by SF2 or SC35 may be explained if hnRNP A1 has a higher affinity for 5′ splice sites than for 3′ splice sites. Once SF7 is identified, it should be possible to determine how all these competing factors influence each other upon binding to pre-mRNA.

Additional pre-mRNAs containing alternative 3′ splice sites should be studied to determine whether the selective effects of SF2 and SC35 are general, and whether the proximal site is always favored, as in the case of alternative 5′ splice sites. The combined stimulation of proximal 5′ and 3′ splice sites by SF2 and SC35 may serve to prevent inappropriate exon skipping (ref. 4; A.M., D. Helfman, and A.R.K., unpublished work). In addition, *in vivo* modulation of the levels or activities of these and other SR proteins, as well as of hnRNP A1 and SF7, may regulate the expression of some of the many genes that are alternatively spliced in a tissue-specific or developmentally controlled manner. Similar studies with other purified general splicing factors should show whether the concentration-dependent polar effects on alternative splice site selection are a unique property of these and/or other SR proteins.

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