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Purification and characterization of a kinase specific for the serine- and arginine-rich pre-mRNA splicing factors

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ABSTRACT **Members of the SR family of pre-mRNA splicing factors are phosphoproteins that share a phosphoepitope specifically recognized by monoclonal antibody (mAb) 104. Recent studies have indicated that phosphorylation may regulate the activity and the intracellular localization of these splicing factors. Here, we report the purification and kinetic properties of SR protein kinase 1 (SRPK1), a kinase specific for SR family members. We demonstrate that the kinase specifically recognizes the SR domain, which contains serine/arginine repeats. Previous studies have shown that dephosphorylated SR proteins did not react with mAb 104 and migrated faster in SDS gels than SR proteins from mammalian cells. We show that SRPK1 restores both mobility and mAB 104 reactivity to a SR protein SF2/ASF (splicing factor 2/alternative splicing factor) produced in bacteria, suggesting that SRPK1 is responsible for the generation of the mAb 104-specific phosphoepitope *in vivo*. Finally, we have correlated the effects of mutagenesis in the SR domain of SF2/ASF on splicing with those on phosphorylation of the protein by SRPK1, suggesting that phosphorylation of SR proteins is required for splicing.**

SR proteins are a family of pre-mRNA splicing factors characterized by the presence of RNA binding motifs and the SR domain, which consists largely of serine/arginine repeats (1). They are essential splicing factors, playing critical roles in the initiation of spliceosome assembly on pre-mRNA substrates (2–6). SR proteins are functionally similar when assayed for splicing *in vitro* (7, 8). However, recent studies have shown that each member displays a distinct spectrum of substrate specificity toward pre-mRNAs (5, 9–11). SR proteins can also alter alternative splice site selection *in vitro* by promoting the use of proximal 5′ and 3′ splice sites in a concentration-dependent manner (8, 10, 12, 13). A series of recent studies have suggested that alternative splice site usage is mediated, at least in part, by direct binding of a SR protein(s) to the site or by recruiting a SR protein(s) to the site by specific alternative splicing regulators (11, 14, 15). Thus, alternative splicing could, in principle, be regulated by controlling the expression and/or activity of SR proteins.

The levels of individual SR proteins have been reported to differ among tissues (10). However, the quantitation of protein expression in this study depends on the use of monoclonal antibody (mAb) 104 that recognizes a specific phosphoepitope present in all SR family members. Thus, the variation in SR proteins detected could reflect the expression levels of SR proteins and/or their differential phosphorylation in different tissues. The SR domain has recently been shown to be responsible for protein–protein interactions during spliceosome assembly (6, 16, 17), and ample potential phosphorylation sites are present in the SR domain. Thus, phosphorylation may modulate SR protein interactions.

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Three lines of evidence suggest that phosphorylation plays a role in pre-mRNA splicing. (*i*) A U1–small nuclear ribonucleoprotein particle (snRNP)-associated kinase activity has been described that could phosphorylate the SR domain in the SR family member splicing factor 2/alternative splicing factor (SF2/ASF) and a similar motif consisting of serine/arginine repeats in the U1 70-kDa protein (18). Incorporation of a nonhydrolyzable homolog of ATP into the isolated U1 snRNP by the kinase impaired its ability to complement splicing in a U1 snRNP-depleted nuclear extract (19). (*ii*) It has been reported (20) that inhibitors to phosphatases can specifically inhibit splicing *in vitro*, suggesting that dephosphorylation is required for splicing. (*iii*) We have identified and cloned (21) a cell-cycle-regulated kinase specific for SR proteins, SR protein kinase 1 (SRPK1), and a high level of this kinase can inhibit splicing. Thus, these studies show that dephosphorylation is important for splicing. However, whether or not phosphorylation of SR proteins and other splicing factors containing a similar SR domain is essential for splicing and the possibility that phosphorylation and dephosphorylation of these proteins occur during different stages of splicing remain to be addressed.

In this paper, we report the purification and characterization of SRPK1. We show that the phosphoepitope on SR proteins specifically recognized by mAb 104 can be restored by SRPK1 to a bacterially produced SR protein, SF2/ASF, suggesting that SRPK1 is responsible for phosphorylation of SR proteins *in vivo*. Finally, we provide evidence that phosphorylation of SR proteins by SRPK1 is also important for splicing because highly conservative changes in the SR domain of SF2/ASF, which were previously shown (23) to abolish its function in splicing, also affected the recognition and phosphorylation of this splicing factor by SRPK1. Further investigation of phosphorylation regulation of splicing will be facilitated by the availability of SRPK1.

MATERIALS AND METHODS

Expression and Quantitation of Recombinant SR Proteins. We initially used SR protein SC35 as a substrate for SRPK1 kinase activity during purification. SC35 was expressed in a baculovirus system and purified as described (5). This protein was further purified on a C_4 HPLC column for the kinase assay (21). Since the SR protein SF2/ASF could be expressed in bacteria as an unphosphorylated form, a large amount of the protein was purified for the kinase assay from inclusion bodies as described (22). Wild-type and mutant SF2/ASF proteins purified from bacteria (23) were kindly provided by J. Caceres and A. Krainer (Cold Spring Harbor Laboratory). We also prepared wild-type SF2/ASF from

Abbreviations: SR family, serine- and arginine-rich phosphoprotein family; SRPK1, SR protein kinase 1; SF2, splicing factor 2; ASF, alternative splicing factor; DTT, dithiothreitol; MBP, myelin basic protein; AS, ammonium sulfate.

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bacteria and quantitated it as follows. The protein purified from inclusion bodies was dialyzed against 50 mM Tris·HCl, pH 7.6/500 mM NaCl/1 mM dithiothreitol (DTT)/0.2 mM EDTA. The protein forms aggregates during dialysis. The aggregates were pelleted, washed once with water, resuspended in water, and lyophilized. Since accurate quantitation of protein concentration is essential for the determination of kinetic values and for the calculation of phosphorylation ratios, purified SF2/ASF was quantitated by protein composition analysis at the Harvard Microchemistry facility. This SF2/ASF preparation was used as a standard for quantitation of other SR proteins by the Bradford assay (Bio-Rad). Myelin basic protein (MBP) was purchased from Sigma.

Kinase Reactions and Determination of Kinetic Parameters. Purified SC35 (0.5 μg) or SF2/ASF (0.5 μg) was used as a substrate in a 20-μl kinase assay mixture (50 mM Tris·HCl, pH 7.4/10 mM $MgCl_2$/1 mM DTT/2 μM ATP/2 μCi of [γ-^{32}P]ATP). For each fraction, the reaction was initiated by adding 2 μl of the column fraction and incubated at room temperature for 15 min. Proteins were resolved in a 12.5% SDS gel. After staining and autoradiography, gel slices containing radioactive proteins were quantitated in a liquid scintillation counter. One unit of SRPK1 activity was defined as 1 pmol of phosphate transferred per min under standard kinase reaction conditions. To measure stoichiometry, 100 μM ATP and saturating SRPK1 (5 units) were used to phosphorylate wild-type and mutant SF2/ASF proteins (0.25 μM or 5 pmol) at 30°C for 2 h. To determine the kinetics of SRPK1, phosphorylation of SF2/ASF was kept linear with respect to reaction time (15 min) and the kinase (1 unit). To determine the K_m for ATP, six concentrations of ATP (1.25 μM, 2.5 μM, 5 μM, 25 μM, 50 μM, and 250 μM) were titrated against four concentrations of SF2/ASF (0.05 μM, 0.1 μM, 0.25 μM, and 0.7 μM). The kinase activity (pmol/min) was measured at each point. The reciprocal kinase activities (1/units) were plotted against the reciprocal ATP concentrations (1/[ATP]) (Lineweaver–Burk plot). For comparison, we only used one constant concentration of ATP (100 μM) and kinase (1 unit) to determine apparent K_m and relative V_{max} values for SF2/ASF and MBP. In this experiment, kinase activities were determined at five concentrations of SF2/ASF (0.05 μM, 0.1 μM, 0.25 μM, 0.5 μM, and 1.0 μM) and four concentrations of MBP (2.5 μM, 5.0 μM, 12.5 μM, and 25 μM).

Purification Procedures. S100 extracts were prepared as described (24) and used for large-scale purifications. All procedures were carried out at 4°C. After removing particles by a low-speed centrifugation, the extract (≈15 mg/ml) was loaded at the speed of 1 ml/min directly onto a 20-ml phosphocellulose P11 (Whatman) column equilibrated in buffer A (20 mM Tris·HCl, pH 7.2/5 mM β-glycerophosphate/1 mM EGTA/1 mM DTT/1.5 mM $MgCl_2$) containing 50 mM NaCl. After washing the column with 40 ml of buffer A containing 50 mM NaCl, the column was developed with a linear gradient from 50 mM to 1 M NaCl. Fractions (10 ml) were collected and 2 μl from each fraction was assayed for the kinase activity using baculovirus-produced SC35 or bacterially produced SF2/ASF (SR proteins were found exchangeable as substrates for the kinase assay during the purification of SRPK1; data not shown). One major kinase peak was identified, and the peak fractions were pooled. Ammonium sulfate (AS) was added to the pooled fractions to 1.5 M. After stirring for 1 h, the precipitate was removed by centrifugation at 8000 rpm in a Sorvall ss34 rotor for 15 min. The supernatant was diluted with buffer B (25 mM Tris·HCl, pH 7.8/1 mM EGTA/1 mM DTT) to bring the AS to 1.25 M and directly loaded at 1 ml/min onto a 1-ml Phenyl-Sepharose (high performance) column (Pharmacia) equilibrated in buffer B containing 1.25 M AS. The column was developed with a linear gradient from 1.25 M AS in buffer B to buffer B only. Fractions from the kinase peak were pooled, diluted 1:10 with buffer B, and loaded directly on a FPLC Mono Q column equilibrated in buffer B plus 50 mM NaCl. The column was developed with a linear gradient from 50 mM to 1 M NaCl in buffer B. Fractions from the kinase peak were pooled, diluted 1:10 with buffer C (30 mM Mes·NaOH, pH 6.2/1 mM EGTA/1 mM DTT), and loaded directly onto a FPLC Mono S column equilibrated in buffer C plus 50 mM NaCl. The column was developed with a linear gradient from 50 mM to 1 M NaCl in buffer C. The kinase-containing fractions were analyzed by SDS/PAGE followed by silver staining.

RESULTS

Purification of SRPK1, a Specific Kinase for SR Proteins. The SRPK1 activity was initially identified in mitotic extracts and shown to be a kinase specific for the SR family of proteins (21). In a search for material for large-scale purification, we found that the S100 extract (accumulated in our freezer as a byproduct of nuclear extract preparation) contained a high level of the kinase activity after the phosphocellulose P11 fractionation step. As shown in Fig. 1*A* and Table 1, the kinase was activated ≈18-fold, whereas the total protein concentration decreased by a factor of ≈50, achieving 900-fold purification in a single step. A similar observation was also made with whole cell extracts derived from mitotically arrested and unsynchronized HeLa cells (data not shown, see ref. 21). The kinases purified from both sources displayed similar effects on splicing and the localization of splicing factors (21). This observation indicates that S100 may contain an inhibitor(s) to SRPK1 and that S100 and whole cell extracts provided sufficient material for large-scale purification.

After testing many column resins and purification conditions, we developed a protocol to purify SRPK1 to homogeneity through four consecutive fractionation steps. The column profiles are shown in Fig. 1 and the purification is summarized in Table 1. At each step, a single kinase peak, assayed using SC35 as a substrate, was identified. SRPK1 bound to a phosphocellulose P11 column and was eluted at 0.7 M NaCl (Fig. 1*A*). The kinase fractions were further fractionated with AS and the supernatant was loaded onto a Phenyl-Sepharose column. The fold purification shown in Table 1 was derived from both AS fractionation and separation on the Phenyl-Sepharose column. At this point, a 92-kDa band that was detected among many other proteins in the SRPK1 peak was eluted at 0.6 M AS (Fig. 1*B*). Further

Table 1. Summary of SRPK1 purification from HeLa cell S100 extracts

Step	Total protein, mg	Total activity, units × 10^{-3}	Specific activity, pmol per min per mg	Fold purification	Recovery, %
S100	1250	1.25	1	1	100
P11	24	21.6	900	900	100
PS	2.0	5.2	2.6×10^3	2,600	24.0
Mono Q	0.20	3.6	1.8×10^4	18,000	17.0
Mono S	0.015	3.5	2.3×10^5	230,000	16.0

PS, Phenyl-Sepharose. One unit of SRPK1 activity is defined as 1 pmol of phosphate transferred per min at room temperature. Specific activity is pmol of phosphate transferred per mg of protein per min.

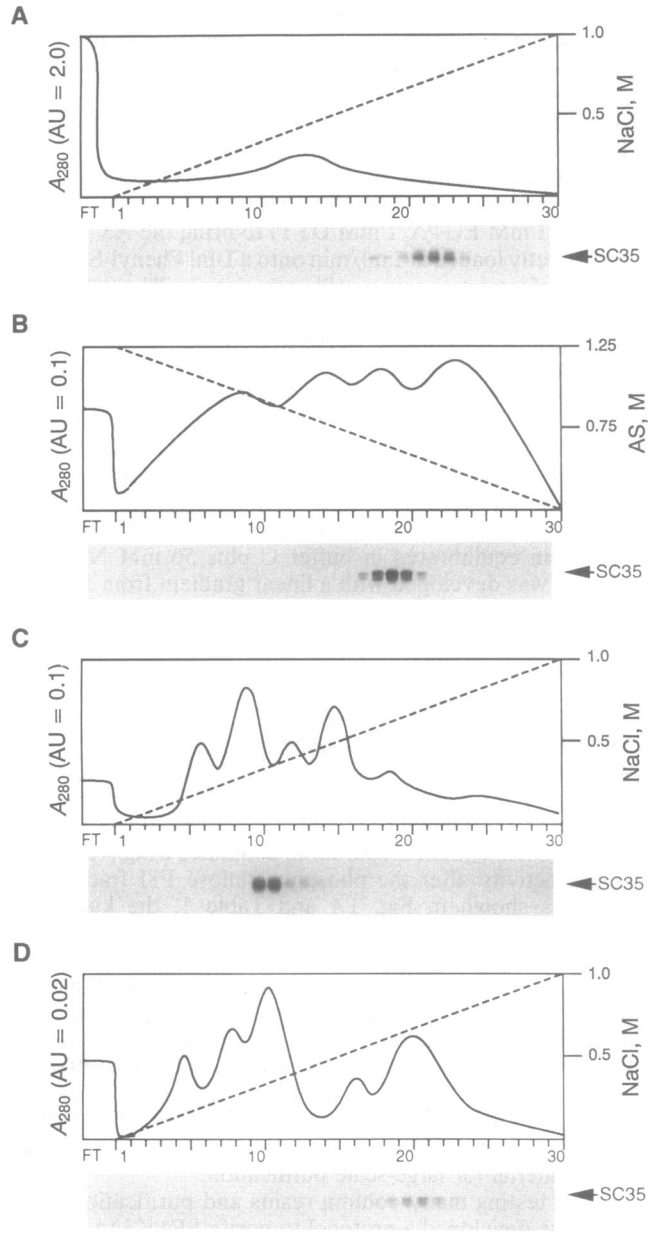

FIG. 1. Purification of SRPK1. S100 extracts were fractionated through four consecutive columns. (*A*) Phosphocellulose P11. (*B*) Phenyl-Sepharose. (*C*) Mono Q. (*D*) Mono S. Total protein (A_{280}, solid lines) and elution gradients (dashed lines) are shown. The results of kinase assay using SC35 as a substrate are included below each column profile. AU, absorbent unit(s); FT, flow through.

fractionation on a Mono Q column revealed that a 92-kDa protein copurified with the SRPK1 activity that was eluted at 0.35 M NaCl (Fig. 1*C*), whereas a contaminating 92-kDa protein was removed (data not shown). Final purification was achieved on a Mono S column where the SRPK1 activity was eluted at 0.75 M NaCl (Fig. 1*D*). The protein profiles at each step of purification are shown in Fig. 2*A*. A single silver-stained protein migrating at 92 kDa was detected, corresponding to the SRPK1 activity as shown in Fig. 2*B*. The protein band was eluted from the SDS gel, and after denaturation and renaturation steps, the eluted protein contained active SRPK1 (data not shown). Partial amino acid sequences were obtained from the purified protein, revealing that SRPK1 is a distinct kinase as reported (21).

Kinetic Properties of SRPK1. We have shown (21) that SRPK1 is specific for splicing factors containing a SR do-

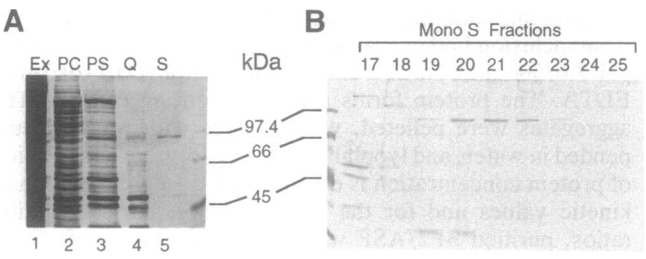

FIG. 2. Protein composition of the kinase-containing peak fractions. (*A*) Proteins in pooled kinase containing fractions from each column were resolved in a 10% SDS gel and silver stained. (*B*) Protein profile of the Mono S fractions across the SRPK1 activity peak. A single 92-kDa protein copurified with the kinase activity.

main. To further characterize the kinase, we carried out kinetic analyses. By using bacterially expressed SF2/ASF as a substrate, SRPK1 exhibited a linear activity at 30°C for ≈1 h (data not shown). The kinase activity depended on ATP concentration with a K_m value for ATP of 10 μM (Fig. 3*A*). This value indicated that the ATP concentration in our standard kinase assay (2 μM ATP) was suboptimal and allowed us to maximize the kinase activity to observe the effect of phosphorylation on the properties of its substrates (see below).

Although SRPK1 is specific for SR proteins, it was possible that other substrates for the kinase exist. Sequence comparison revealed that SRPK1 is highly related to a fission yeast cell-cycle-regulated kinase, dsk1 (21, 25). Although the function of dsk1 is not yet defined, it was shown to phosphorylate MBP *in vitro*. We therefore tested MBP as a substrate for SRPK1 and compared it with the SR protein SF2/ASF. Using an optimal ATP concentration (100 μM), we found that MBP could indeed be phosphorylated, but the difference between MBP and SF2/ASF was dramatic (Fig. 3*B*). We calculated apparent K_m and relative V_{max} values and the phosphorylation ratios for both substrates. The results showed that SRPK1 has a K_m value of 0.07 μM and a V_{max} value of 0.45 pmol per min per unit of kinase when tested with SF2/ASF, indicating that SRPK1 has a very high affinity for SR proteins (Fig. 3*B*). In contrast, SRPK1 displayed a high K_m value of

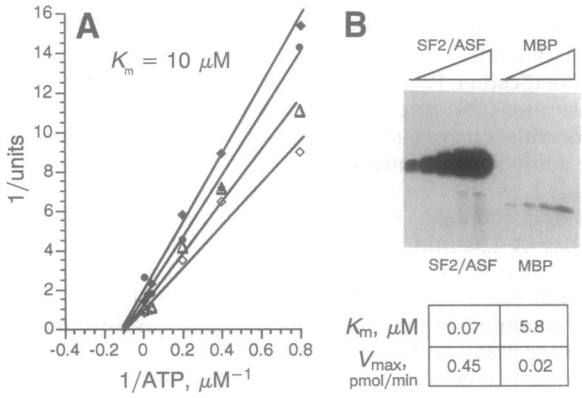

FIG. 3. Kinetic properties of SRPK1. (*A*) Determination of the K_m value for ATP. Various ATP concentrations (1.25 μM, 2.5 μM, 5 μM, 25 μM, 50 μM, and 250 μM) were titrated against four SF2/ASF concentrations (◆, 0.05 μM; ●, 0.1 μM; △, 0.25 μM; ◇, 0.7 μM). Double-reciprocal plots are shown. The K_m value for ATP was calculated to be 10 μM. Unit(s) are as defined in Table 1. (*B*) Phosphorylation of SF2/ASF and MBP by SRPK1. Five SF2/ASF concentrations (0.05 μM, 0.1 μM, 0.25 μM, 0.5 μM, and 1.0 μM) and four MBP concentrations (2.5 μM, 5.0 μM, 12.5 μM, and 25 μM) were used in the phosphorylation reactions. Saturating ATP (100 μM) and a constant amount of SRPK1 (1 unit) were used in each reaction mixture. The apparent K_m and relative V_{max} values calculated based on these data are shown at the bottom.

5.8 μM, and very low V_{max} value of 0.02 pmol per min per unit of kinase for MBP (Fig. 3*B*). In addition, while several phosphates were transferred to one SF2/ASF molecule (see below), <1 out of 100 MBP molecules was phosphorylated. These data suggest that SR proteins, rather than MBP, are physiological substrates for SRPK1, and possibly dsk1.

SRPK1 Restores a Specific Phosphoepitope on Recombinant SF2/ASF. To prove that SRPK1 is responsible for phosphorylation of SR proteins requires that, as shown by phosphopeptide mapping, the same sites in SR proteins are phosphorylated in cells and *in vitro* by the isolated kinase. Despite the fact that SF2/ASF has a relatively small SR domain compared to other SR proteins, it contains 16 potential phosphorylation sites in SR repeats. It is therefore technically difficult to map the actual sites. To overcome this problem, we took advantage of the observation that the phosphoepitopes present in SR proteins from mammalian cells are specifically recognized by mAb 104 (26). As demonstrated by Roth and his colleagues (1, 26), mAb 104 reacted with all members of the SR family. Treatment of any SR protein with a phosphatase resulted in an increase in its mobility in SDS gels and in loss of its reactivity with mAb 104 (data not shown, see ref. 26). We therefore used the mobility shift and reactivity to mAb 104 as indicators to test whether the specific phosphoepitopes could be restored in a bacterially expressed SR protein by purified SRPK1. As shown in Fig. 4, under optimal conditions, phosphorylation of bacterially produced SF2/ASF by SRPK1 resulted in a marked mobility shift in a SDS gel (Fig. 4*A*), and furthermore, the *in vitro*-phosphorylated SF2/ASF was now reactive to mAb 104 (Fig. 4*B*). These results demonstrate that SRPK1 is responsible for the formation of the specific phosphoepitope present in native SF2/ASF, suggesting that SRPK1 phosphorylates SR proteins *in vivo*.

Conservative Mutations in the SR Domain Abolish Both Splicing Function and SRPK1 Recognition. Recent studies demonstrated that the SR domain in SF2/ASF is essential for splicing (23, 27). Caceres and Krainer (23) have shown that replacements of serine with threonine or of arginine with lysine in the SR domain abolished the splicing activity of SF2/ASF (Fig. 5*A* and ref. 23). Since these conservative changes would not be expected to alter protein conformation, it is possible that phosphorylation on serines as signaled by arginines is required for the function of SF2/ASF. To test this possibility and to further characterize purified SRPK1, we tested phosphorylation of mutant SF2/ASF proteins by SRPK1 (Fig. 5 *B* and *C*).

We observed that eight or nine phosphates could be transferred to one molecule of wild-type SF2/ASF under optimal conditions. Deletion of the serine/arginine repeats abolished phosphorylation, as shown (21). Interestingly,

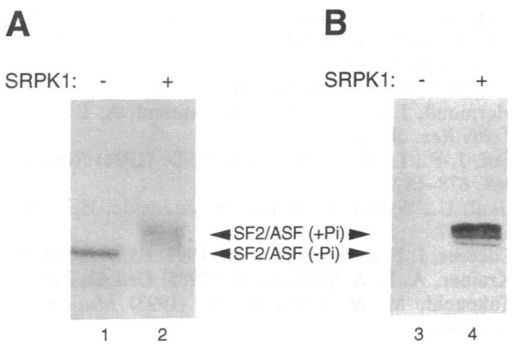

FIG. 4. Phosphorylation of bacterially expressed SF2/ASF by SRPK1 resulted in a mobility shift in a 12.5% SDS gel (*A*) and a gain of reactivity to mAb 104 (*B*). Lanes: 1 and 3, bacterially expressed SF2/ASF; 2 and 4, SRPK1-phosphorylated SF2/ASF.

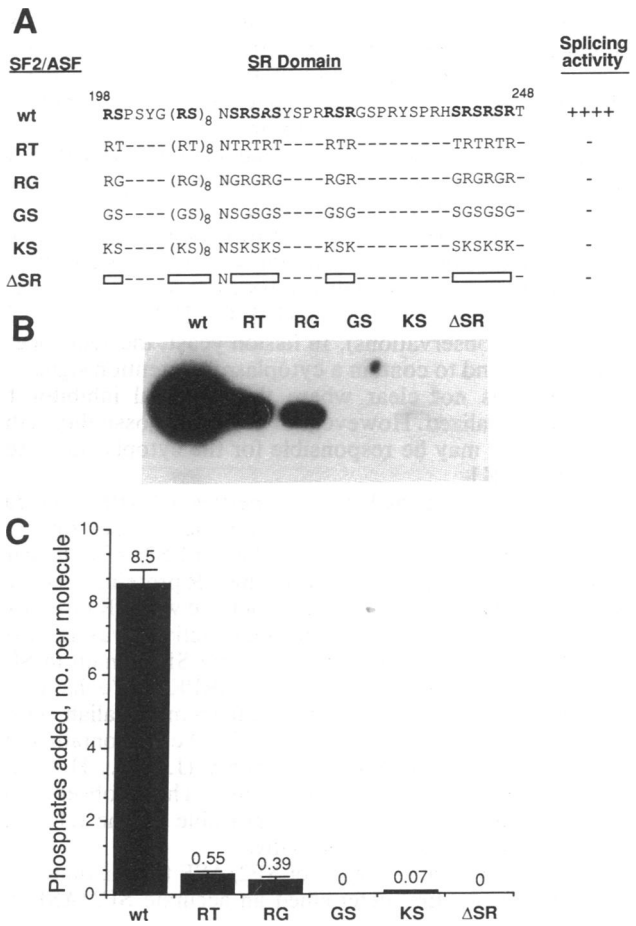

FIG. 5. Phosphorylation of wild-type and mutant SF2/ASF by SRPK1. (*A*) Effects of the mutations in the SR domain of SF2/ASF on splicing. Sequences and effects of mutations on splicing are from Caceres and Krainer (23). wt, Wild type. (*B*) Effects of the mutations in the SR domain of SF2/ASF on phosphorylation by SRPK1. (*C*) Quantitation of phosphorylation by SRPK1 of wild-type and mutant SF2/ASF proteins. The data are from three experiments and expressed as number of phosphates transferred per molecule of SF2/ASF.

when the serine residues were replaced with glycine, maximally, one phosphate could still be transferred to one molecule of the mutant protein. We believe that this phosphorylation took place on one of four serine residues outside the serine/arginine repeats (see Fig. 5*A*). A similar observation was made with the serine → threonine substitution, indicating that threonine could not replace serine as a recipient for phosphorylation by SRPK1. When arginines were replaced with glycines, phosphorylation was completely inhibited. This result clearly showed that arginine is important for signaling phosphorylation both within and outside the serine/arginine repeats. Interestingly, arginine could not be functionally replaced by lysine. Therefore, mutations that cripple the function of SF2/ASF in splicing also result in diminished phosphorylation, strongly suggesting that phosphorylation of SR proteins by SRPK1 is essential for splicing.

DISCUSSION

In this report, we have purified and characterized SRPK1 derived from S100 extracts. Since the S100 extract contains all splicing components except SR proteins (2), the cellular distribution of SRPK1 remains to be investigated. A comparable level of SRPK1 could also be detected in splicing nuclear extracts (H.T., J.-F.G., and X.-D.F., unpublished results), suggesting that at least a fraction of the kinase must

be present in the nucleus. Consistent with this idea, two potential nuclear-targeting signals are present in the sequence from a cDNA clone for SRPK1 (21). Interestingly, its fission yeast homologue, dsk1, was found to be mainly distributed in the cytoplasm in interphase cells and migrate to the nucleus before mitosis (25). If this is also characteristic of SRPK1, its nuclear translocation may be a critical step in the regulation of pre-mRNA splicing in interphase cells.

Activation of SRPK1 during purification suggests that a potential inhibitor(s) has been removed. Preliminary studies indicate that such an inhibitor does exist and can be recovered from a single FPLC peak (J.-F.G., H.T., and X.-D.F., unpublished observations). In fission yeast, the sequence of dsk1 was found to contain a cytoplasmic retention signal. At present, it is not clear where the potential inhibitor for SRPK1 is localized. However, an intriguing possibility is that this inhibitor may be responsible for the cytoplasmic retention of SRPK1.

In characterizing the kinetic properties of SRPK1, the data suggest that SR proteins are physiological substrates for SRPK1. The extremely low K_m value for SF2/ASF indicates that SRPK1 has a high affinity for this SR protein, raising the possibility that SRPK1 may be associated with SR proteins *in vivo*. Recently, a U1-associated kinase activity was described (18) that could phosphorylate the same SR domain in SF2/ASF that was phosphorylated by SRPK1. We have also observed that SR proteins purified from mammalian cells or expressed from baculovirus-infected Sf9 cells contain a high level of an associated kinase activity (J.-F.G., H.T., and X.-D.F., unpublished observations). These observations suggest that SRPK1 could be responsible for the U1- or SR protein-associated kinase activities.

To use a calibrated sample of SF2/ASF to quantitate phosphorylation, we first determined an accurate SF2/ASF concentration by purifying the bacterially expressed protein to homogeneity followed by protein composition analysis. Within the SR domain of SF2/ASF, there are 16 serines within the SR repeats and 4 serines outside the repeats (Fig. 5*A*). Even if we may have overestimated the protein concentration of SF2/ASF, our results suggest that at least half of the serine residues within the SR repeats can be phosphorylated. Phosphorylation of the SR domain would change the ratio of positive and negative charges, a consequence that may be important for protein folding, RNA binding, and/or protein–protein interactions during splicing and spliceosome assembly.

Roth and his coworkers (1, 26) have shown that phosphorylation of SR proteins is correlated with their mobility on SDS gels and their reactivity to mAb 104. We have demonstrated that both mobility on SDS gels and reactivity to mAb 104 could be restored to a bacterially expressed SR protein by purified SRPK1, indicating that SRPK1 is responsible for phosphorylation of SR proteins *in vivo*. We do not know the minimal number and location of phosphoserines in the SR domain that are important for the mobility shift and reactivity to mAb 104. Therefore, the possibility remains that there are redundant kinases in mammalian cells. In fission yeast, overexpression of dsk1 caused a cell-cycle-deficient phenotype, e.g., a delay in the G_2- to M-phase transition (25). However, disruption of the dsk1 gene had no effect on viability or growth, suggesting the existence of functionally redundant kinases. Interestingly, only one splicing factor containing a SR domain, prp2, a homologue of human U2AF, has been isolated from *Schizosaccharomyces pombe*, but not yet from *Saccharomyces cerevisiae* (28). Whether the prp2 product from *S. pombe* is a substrate for dsk1 and SRPK1 remains to be determined. If SRPK1 can phosphorylate prp2, additional yeast substrates containing a SR-like domain from both *S. cerevisiae* and *S. pombe* may be detectable biochemically, which otherwise might have failed to be isolated genetically because of their redundancy.

We demonstrated in this paper that the effects of conservative changes within the SR domain of SF2/ASF on splicing can be accounted for by the effects of these changes on phosphorylation by SRPK1, arguing strongly that phosphorylation is important for the function of SR proteins in splicing. In our report (21), we also showed that a high level of SRPK1 was inhibitory to splicing. Thus, these data suggest that the function of SR proteins in splicing may be regulated by phosphorylation and dephosphorylation. The SR domain has been shown to participate in protein–protein interactions (6, 16, 17). Thus, phosphorylated SR proteins may be required to mediate steps during spliceosome assembly, and dephosphorylation of these SR proteins may be essential for the activation and resolution of spliceosome. Identification and purification of SRPK1, a kinase specific for SR proteins, will have an impact on refining this model and understanding the regulation of pre-mRNA splicing.

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1. Zahler, A. M., Lane, W. S., Stalk, J. A. & Roth, M. B. (1992) *Genes Dev.* **6**, 837–847.
2. Krainer, A. R., Conway, G. C. & Kozak, D. (1990) *Genes Dev.* **4**, 1158–1171.
3. Fu, X.-D. & Maniatis, T. (1990) *Nature (London)* **343**, 437–441.
4. Fu, X.-D. & Maniatis, T. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1725–1729.
5. Fu, X.-D. (1993) *Nature (London)* **365**, 82–85.
6. Kohtz, J. D., Jamison, S. F., Will, C. L., Zuo, P., Luhrmann, R., Garcia-Blanco, M. A. & Manley, J. L. (1994) *Nature (London)* **368**, 119–124.
7. Mayeda, A., Zahler, A. M., Krainer, A. R. & Roth, M. B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1301–1304.
8. Fu, X.-D., Mayeda, A., Maniatis, T. & Krainer, A. R. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 11224–11228.
9. Kim, Y.-J., Zuo, P., Manley, J. L. & Baker, B. S. (1992) *Genes Dev.* **6**, 2569–2579.
10. Zahler, A. M., Neugebaur, K. M., Lane, W. S. & Roth, M. B. (1993) *Science* **260**, 219–222.
11. Tian, M. & Maniatis, T. (1993) *Cell* **74**, 105–114.
12. Ge, H. & Manley, J. L. (1990) *Cell* **62**, 25–34.
13. Krainer, A. R., Conway, G. C. & Kozak, D. (1990) *Cell* **62**, 35–42.
14. Sun, Q., Mayeda, A., Hampson, R. K., Krainer, A. R. & Rottman, F. M. (1993) *Genes Dev.* **7**, 2598–2608.
15. Lavigueur, A., Branche, H. L., Kornblihtt, A. R. & Chabot, B. (1993) *Genes Dev.* **7**, 2405–2417.
16. Amrein, H., Hedley, M. & Maniatis, T. (1994) *Cell* **76**, 735–746.
17. Wu, J. Y. & Maniatis, T. (1993) *Cell* **75**, 1061–1070.
18. Woppmann, A., Will, C. L., Kornstadt, U., Zuo, P., Manley, J. L. & Luhrmann, R. (1993) *Nucleic Acids Res.* **21**, 2815–2822.
19. Tazi, J., Kornstadt, U., Rossi, F., Jeanteur, P. & Luhrmann, R. (1993) *Nature (London)* **363**, 283–286.
20. Mermoud, J. E., Cohen, P. & Lamond, A. I. (1992) *Nucleic Acids Res.* **20**, 5263–5269.
21. Gui, J.-F., Lane, W. S. & Fu, X.-D. (1994) *Nature (London)* **369**, 678–682.
22. Gaul, U., Seifert, E., Schuh, R. & Jackle, H. (1987) *Cell* **50**, 639–647.
23. Caceres, J. F. & Krainer, A. R. (1993) *EMBO J.* **12**, 4715–4726.
24. Krainer, A. R. & Maniatis, T. (1985) *Cell* **42**, 725–736.
25. Takeuchi, M. & Yanagida, M. (1993) *Mol. Biol. Cell.* **4**, 247–260.
26. Roth, M. B., Zahler, A. M. & Stolk, J. A. (1991) *J. Cell Biol.* **115**, 587–596.
27. Zuo, P. & Manley, J. L. (1993) *EMBO J.* **12**, 4727–4737.
28. Potashkin, J., Naik, K. & Wentz-Hunter, K. (1993) *Science* **262**, 573–575.