doi:10.1016/j.jmb.2007.08.029 J. Mol. Biol. (2008) 376, 55–68

JMB

Available online at www.sciencedirect.com

ScienceDirect

ELSEVIER

Ordered Multi-site Phosphorylation of the Splicing Factor ASF/SF2 By SRPK1

Chen-Ting Ma[1], Adolfo Velazquez-Dones[2], Jonathan C. Hagopian[1] Gourisankar Ghosh[2], Xiang-Dong Fu[3] and Joseph A. Adams[1]*

[1]*Department of Pharmacology, University of California, San Diego, La Jolla, CA 92093-0636, USA*

[2]*Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92093, USA*

[3]*Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093, USA*

Received 11 July 2007; received in revised form 13 August 2007; accepted 15 August 2007
Available online 21 August 2007

Edited by D. E. Draper

The human alternative splicing factor ASF/SF2, an SR (serine–arginine-rich) protein involved in mRNA splicing control, is activated by the multisite phosphorylation of its C-terminal RS domain, a segment containing numerous arginine–serine dipeptide repeats. The protein kinase responsible for this modification, SR-specific protein kinase 1 (SRPK1), catalyzes the selective phosphorylation of approximately a dozen serines in only the N-terminal portion of the RS domain (RS1). To gain insights into the nature of selective phosphate incorporation in ASF/SF2, region-specific phosphorylation in the RS domain was monitored as a function of reaction progress. Arg-to-Lys mutations were made at several positions to produce unique protease cleavage sites that separate the RS domain into identifiable N- and C-terminal phosphopeptides upon treatment with lysyl endoproteinase. These studies reveal that SRPK1 docks near the C-terminus of the RS1 segment and then moves in an N-terminal direction along the RS domain. Multiple quadruple Ser-to-Ala and deletion mutations did not disrupt the phosphorylation of other sites regardless of position, suggesting that the active site of SRPK1 docks in a flexible manner at the center of the RS domain. Taken together, these data suggest that SRPK1 uses a unique 'grab-and-pull' mechanism to control the regiospecific phosphorylation of its protein substrate.

Keywords: kinase; kinetics; phosphorylation; splicing; SR protein

The splicing of precursor mRNA is an essential chemical transformation to generate mature mRNA for protein translation. While some genes are constitutively spliced, many are alternatively spliced where the selection of 5′ and 3′ splice sites is regulated.[1] Alternative splicing can generate multiple proteins from a single gene and provide a means for the amplification and regulation of the genome in a cell- and tissue-specific manner.[2] It is now thought that as much as 74% of human genes have at least two splice variants, underscoring the pervasiveness of alternative splicing as a source of genetic complexity.[3] Splicing occurs in a macromolecular complex (spliceosome) composed of several small RNA molecules [U1–U6 small nuclear ribonucleoprotein particles (snRNPs)] and numerous protein components. The snRNPs that constitute the core machinery of the spliceosome bind in sequential fashion to pre-mRNA, undergoing several large conformational changes before a fully active spliceosome develops.[4] In addition to snRNPs, the spliceosome contains many non-snRNPs that are functionally important. A family of splicing factors known as SR proteins (splicing factors containing arginine–serine repeats) has been identified for their important role in committing a pre-mRNA to the splicing pathway mediating spliceosome assembly at multiple steps.[5] SR proteins are composed of one or two RNA recognition motifs (RRMs) and a C-terminal RS domain, a segment rich in numerous arginine–serine dipeptide repeats.[6] The prototypical SR protein ASF/SF2 (human alternative splicing factor) con-

*Corresponding author. E-mail address: joeadams@chem.ucsd.edu.

Abbreviations used: ASF/SF2, human alternative splicing factor; GSK-3, glycogen synthase kinase-3; LysC, lysyl endoproteinase; MALDI-TOF, matrix-assisted laser desorption ionization–time of flight; RRM, RNA recognition motif; RS domain, domain rich in arginine–serine repeats; SR protein, splicing factor containing arginine–serine repeats; SRPK1, SR-specific protein kinase 1; TFA, trifluoroacetic acid; wt, wild type.

0022-2836/$ - see front matter. Published by Elsevier Ltd.

tains two RRMs followed by a 50-residue RS domain (Fig. 1a).

Phosphorylated SR proteins have been shown to play a role in modulating protein–protein and protein–RNA interactions within the spliceosome. For example, SR proteins interact with the RS domains of U1-70K (a component of the U1 snRNP) and the U2AF heterodimer, which are thought to be critical for the selection of both 5′ and 3′ splice sites.[7–9] While some SR proteins reside exclusively within the nucleus, others can shuttle between the nucleus and the cytoplasm, where they may be important for multiple processes in RNA metabolism beyond splicing. For example, in hypophosphorylated forms, the shuttling SR proteins ASF/SF2 and 9G8 interact with the nuclear export protein TAP, suggesting that dephosphorylation of SR proteins may allow coupling between splicing and downstream events.[10–12] Reentry of the SR protein into the nucleus is dependent on the actions of SR-specific protein kinase 1 (SRPK1). Once inside the nucleus, SR proteins are likely to be further modified by Clk/Sty, the prototype for the Clk family of nuclear kinases. Although the precise function of Clk kinases is not well understood, it is thought that these enzymes play a role in recruiting SR proteins from nuclear storage sites (speckles) to splicing sites.[13,14] Recent findings also suggest that speckles are a hub of activity for RNA processing, with splicing and transcription occurring toward the periphery of these nuclear compartments and with mature mRNA being stored within the speckles prior to export to the cytoplasm.[15]

In prior work, we have shown that SRPK1 forms an unusually stable complex with ASF/SF2 ($K_d \sim$ 50 nM) and then catalyzes the phosphorylation of

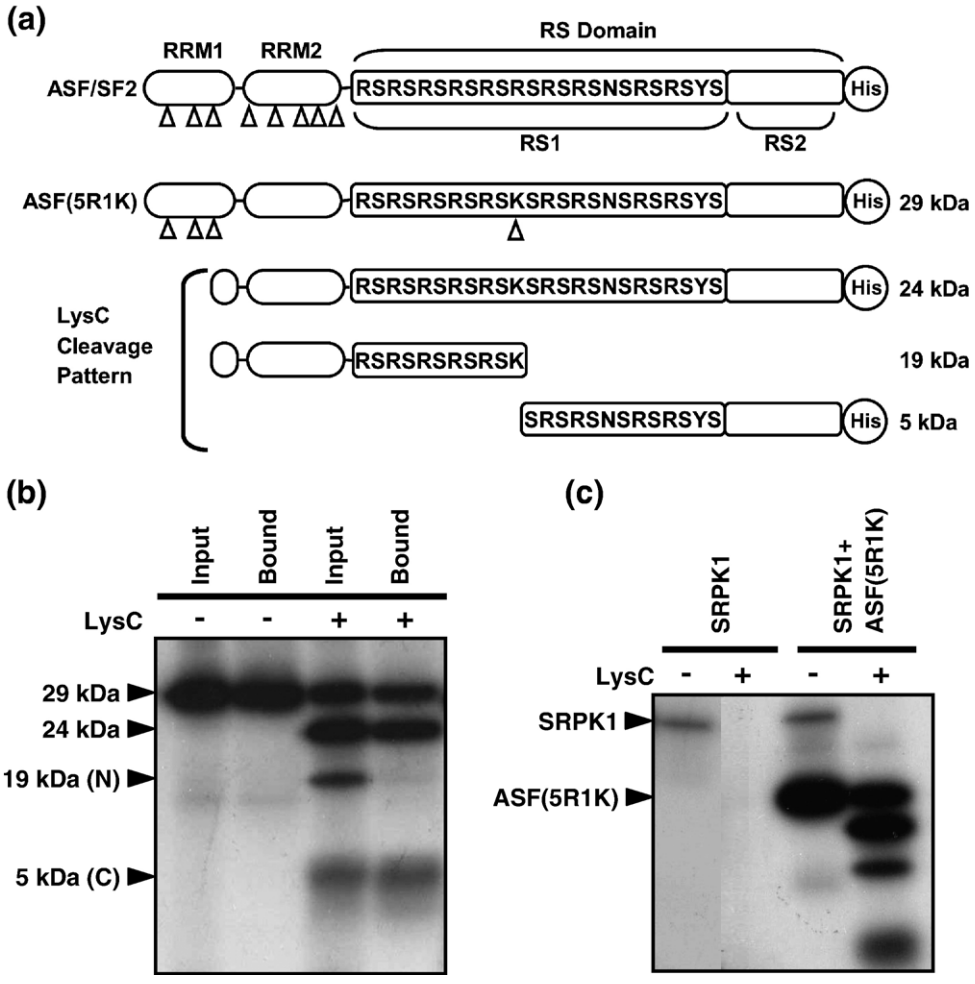

Fig. 1. wt-ASF/SF2 structure and ASF(5R1K) cleavage pattern. (a) Domain organization of wt-ASF/SF2 and expected LysC cleavage fragments of ASF(5R1K). LysC protease cleavage sites are shown using open arrows. An RS domain with complete sequence for RS1 subdomain is shown. The RS2 sequence is $PRRSRGSPRYSPRHSRSRSRT_{248}$. Predicted peptide fragments carrying phosphorylation sites for ASF(5R1K) are shown. (b) Identification of phosphorylated peptide fragments by autoradiography. ASF(5R1K) (250 nM) is phosphorylated using SRPK1 (1 μM) and [^{32}P]ATP (100 μM) for 20 min and then treated with LysC (2 ng/μL) for 4 h at 37 °C before a 1-h incubation with Ni^{2+}-Sepharose resin. After the peptides had been washed, they were eluted from the resin with 50 mM EDTA in SDS-PAGE loading buffer. (c) LysC fragments from ASF(5R1K). The LysC cleavage of SRPK1 (1 μM) with [^{32}P]ATP (100 μM) is carried out in the absence and in the presence of ASF(5R1K), and the products are displayed by autoradiogram.