Abstract. The SR proteins, a group of abundant arginine/serine (RS)-rich proteins, are essential pre-mRNA splicing factors that are localized in the nucleus. These proteins serve as a nuclear localization signal. We found that RS domain–bearing proteins do not utilize any of the known nuclear import receptors and identified a novel nuclear import receptor specific for SR proteins. The SR protein import receptor, termed transportin-SR (TRN-SR), binds specifically and directly to the RS domains of A SF/SF2 and SC35 as well as several other SR proteins. The nuclear transport regulator RanGTP abolishes this interaction. Recombinant TRN-SR mediates nuclear import of RS domain–bearing proteins in vitro. TRN-SR has amino acid sequence similarity to several members of the importin β/transportin family. These findings strongly suggest that TRN-SR is a nuclear import receptor for the SR protein family.

Key words: SR proteins • RS domain • nuclear localization signal • nuclear import receptor • RanGTP
In addition to the two NLSs described above, the arginine/serine rich (RS) domain of SR proteins has been shown also to function as an NLS (Li and Bingham, 1991; Hedley et al., 1995; Cáceres et al., 1997). SR proteins are essential splicing factors, characterized by the presence of at least one RNA-binding domain and a domain containing several, often numerous, arginine/serine dipeptide repeats (for review see Fu, 1995). Several SR proteins have been described including SRp20, 30 (ASF/SF2, SC35), 40, 55, and 75 (Fu and Maniatis, 1990, 1992; Ge and Manley, 1990; K rainer et al., 1990, 1991; Ge et al., 1991; Zahler et al., 1992, 1993). In addition to their roles as essential splicing factors, SR proteins can modulate splice site selection and thus also have important regulatory roles in alternative splicing (Ge and Manley, 1990; Krainer et al., 1990, 1991; Ge et al., 1991; Fu et al., 1992; Zahler et al., 1993; Cáceres et al., 1994). SR proteins are generally found throughout the nucleoplasm and are often particularly concentrated in nuclear speckles, or interchromatin granules (Fu and Maniatis, 1990; Fu, 1995; Cáceres et al., 1997; Singer and Green, 1997; Lamond and Earsnaw, 1998; Misteli and Spector, 1998). Several of the SR proteins have also been shown to shuttle between the nucleus and the cytoplasm (Cáceres et al., 1998) and to accompany mRNA as they are exported through the NPC (A Izhanova-Ericsson et al., 1996).

Although the RS domain has been shown to function as an NLS for SR proteins and to participate in their localization to speckles (Li and Bingham, 1991; Hedley et al., 1995; Cáceres et al., 1997), the nuclear import pathway for SR proteins has not been previously characterized. Here we show that the nuclear import of several of the SR proteins, including A SF/SF2 and SC35, is mediated by a specific import receptor, termed transportin-SR (TRN-SR). TRN-SR is a novel member of the importin β/transportin family and we show that it binds specifically and directly to the RS domains of ASF/SF2, SC35, and to several additional SR proteins. These findings indicate that TRN-SR is the nuclear import receptor for many SR proteins.

**Materials and Methods**

**Construction of Expression Plasmids and Recombinant Protein Preparation**

A fragment corresponding to human A SF/SF2 RS domain (amino acids 198–248) was PCR amplified and inserted into BamHI and Xhol sites in either pGEX-SX-1 (Pharmacia Biotech) or pMal-c2 (New England Biolabs). This fragment was also cloned between EcoRI and SalI sites of pLexA (Clontech, Inc.) for the construction of the yeast two-hybrid library screening bait plasmid. The plasmid encoding human SC35 RS domain amplified by PCR into pGEX-5X-1. The full-length of SC35 RS domain amplified by PCR from clone 1-1 was used as a hybridization probe to screen the HeLa MATCHMAKER LexA cDNA library. Several colonies were isolated, and the clone that had the longest insert was sequenced and thus determined as the full-length coding sequence of TRN-SR.

**Protein-binding Assays**

TRN-SR was produced by in vitro transcription-translation of His-TRN-SR, using a TNT kit (Promega) in rabbit reticulocyte lysate in the presence of [35S]methionine (Amersham) according to the procedure that the manufacturer recommends. Purified recombinant GST and GST fusion proteins (5 μg each) were immobilized on 50 μl of glutathione-Sepharose (Pharmacia) in PBS for at least 1 h at 4°C. The resin was washed with 500 μl of binding buffer (50 mM Tris-HCl, 400 mM NaCl, 5 mM MgOAc, 2 μg/ml of leupeptin, 2 μg/ml of pepstatin, 1% aprotinin, and 0.05% [wt/vol] digitonin; Calbiochem). In vitro translated TRN-SR was added and incubated with these immobilized proteins for 1 h at 4°C. For the experiments to check the effect of exogenous Ran protein, His-tagged RanQ69L (GTP form) was added at a concentration of 2 μM. The resin was washed with 500 μl of binding buffer five times and the bound fraction was eluted by boiling in SDS-PAGE sample buffer. The bound fraction was then analyzed by SDS-PAGE and visualized by fluorography.

**Far Western Blotting with SR Proteins**

Purified SR proteins were kindly provided by Dr. A Kila M ayeda prepared from HeLa cells as described previously (Zahler et al., 1992). 10 μg of proteins was analyzed by SDS-PAGE and transferred to nitrocellulose membrane. Far Western blotting was performed as described previously (Siomi et al., 1997) by using either TRN-SR or TRN1 produced by a TNT kit (Promega) in rabbit reticulocyte lysate in the presence of [35S]methionine (Amersham).

**Results**

**Characterization of SR Protein Nuclear Import**

To characterize the import pathway for SR proteins, we carried out in vitro nuclear import assays in digitonin-permeabilized HeLa cells (A dam et al., 1990). As a substrate we used recombinant GST fused to amino acids 198–248 of A SF/SF2 which corresponds to the RS domain (GST-A SF/SF2 RS) of this protein (G e et al., 1991; K rainer et al., 1995). The Journal of Cell Biology, Volume 145, 1999
Efficient nuclear import of GST-ASF/SF2 RS was observed in the presence of cytosol and an ATP-regenerating system (Fig. 1). As no import was detected without addition of cytosol (Fig. 1), this indicates that nuclear import of ASF/SF2 requires additional soluble factor(s). Efficient nuclear import of GST-ASF/SF2 RS was observed in the presence of an ATP-regenerating system and was reduced by incubation with apyrase (Fig. 1), suggesting a role for NTPs in this process. The import of GST-ASF/SF2 RS was strongly inhibited by RanQ69L, a Ran mutant that cannot hydrolyze GTP at a significant rate (Klebe et al., 1995) (Fig. 1), suggesting a role for RanGTP. GST-ASF/SF2 RS import also has several characteristics of nuclear import that occur through NPCs. Both WGA and an importin β dominant-negative mutant (Impβ ΔN44), reagents which block active nuclear import through NPCs (Forbes, 1992; Görlich et al., 1996b; Kutay et al., 1997a), completely abolished GST-ASF/SF2 RS nuclear import (Fig. 1).

To determine whether a specific and saturable factor(s) participates in RS domain-mediated nuclear import, we tested the effect of excess RS domain on the import of classical NLS, M9, and RS domain–bearing proteins. For these experiments we prepared an MBP fusion of the A SF/SF2 RS domain, termed MBP-RS, as a competitor. Nuclear import assays were carried out in the presence of a 20-fold molar excess of either MBP or MBP-RS. MBP itself had no effect on nuclear import; however, MBP-RS strongly inhibited GST-A SF/SF2 RS import, whereas import of other substrates, GST-SV-40 T NLS, GST-IBB, and GST-M9, was unaffected or only slightly reduced (Fig. 2). These results suggest that a specific nuclear import receptor, distinct from importin β and TRN1, mediates RS domain nuclear import.

**Identification of RS Domain–interacting Proteins**

To identify candidate mediator(s) of SR protein nuclear import, we carried out a yeast two-hybrid screening on a HeLa cell cDNA library using the COOH-terminal 51– amino acid region of ASF/SF2 as bait. This fragment contains the RS domain and is sufficient for complete nuclear localization of myc-tagged pyruvate kinase in HeLa cells (data not shown). Several positive interacting clones were isolated and characterized. One of these, clone 1-1, was isolated four times out of 16 clones, and its deduced amino acid sequence showed significant similarity to that of a putative importin β/transportin-related nuclear transport receptors.

The 1-1 DNA insert was subcloned and used for hybridization screening of a λ phage HeLa cDNA library. A 3-kb clone that appears to contain the entire coding region was obtained. The predicted amino acid sequence of this protein, which we termed TRN-SR, because it turned out, like TRN1, to be a transport receptor of pre-mRNA/mRNA-binding proteins, is shown in Fig. 3. TRN-SR is a 975– amino acid protein with a calculated molecular mass of 1147.
The amino-terminal domain of TRN-SR shows significant sequence similarity to other importin β/transportin family members, including a region required for RanGTP binding (Görlich et al., 1997). The sequence of the original 1-1 clone isolated from the yeast two-hybrid screening starts at amino acid 590 of the TRN-SR sequence and contains the entire COOH-terminal domain. A BLAST homology search with full-length TRN-SR revealed three proteins that bear significant homology to TRN-SR in other species (Fig. 3). The most similar of these, AF025464 of Caenorhabditis elegans, is 26% identical and 45% similar to TRN-SR. Another apparent homologue is AL022304 of Schizosaccharomyces pombe that is 25% identical and 46% similar, although this clone does not appear to contain the full-length protein sequence. These two sequences are the two closest orthologues of TRN-SR present in available databases. Of previously characterized proteins, the most sign-
Significant similarity is found with the S. cerevisiae protein Mtr10p (Kadowaki et al., 1994) which has been shown recently to be a nuclear import receptor for Npl3p (Pemberton et al., 1997; Senger et al., 1998). Npl3p is an hnRNP protein in yeast (Bossie et al., 1992; Russell and Tollervey, 1992; Wilson et al., 1994). The amino acid sequences of TRN-SR and Mtr10p are 21% identical and 42% similar.

**TRN-SR Binds Specifically to the RS Domain of SR Proteins**

To confirm that TRN-SR binds specifically to SR proteins, we carried out in vitro binding experiments using TRN-SR produced by transcription-translation in rabbit reticulocyte lysate. In the same experiments we also tested another RS domain, that of the SR splicing factor SC35 (Fu and Maniatis, 1992). TRN-SR binds to the RS domains of both ASF/SF2 and SC35, but not to IBB or to hnRNP A1 M9 (Fig. 4 A). RanQ69L abolishes the binding of TRN-SR to RS domains (Fig. 4 A), consistent with the possibility that it is a nuclear import receptor for these proteins. Since rabbit reticulocyte lysate contains many proteins, the binding of TRN-SR detected in Fig. 4 A could be indirect. To examine whether TRN-SR can bind to the RS domains directly, we carried out binding assays using purified recombinant TRN-SR. As shown in Fig. 4 B, bacterially expressed TRN-SR binds to both GST-ASF/SF2 RS and GST-SC35 RS, but not to GST alone. These results strongly suggest that TRN-SR is a specific import receptor for SR proteins.

**TRN-SR Mediates the Nuclear Import of RS Domain–containing Proteins**

To determine if TRN-SR is the nuclear import receptor of SR proteins, recombinant TRN-SR was used in in vitro nuclear import assays using either GST-ASF/SF2 RS or GST-SC35 RS as a substrate. Neither GST-ASF/SF2 RS nor GST-SC35 RS by itself accumulated in the nucleus (Fig. 5). However, in the presence of ATP, an ATP-regenerating system and RanGDP, TRN-SR efficiently imported GST-ASF/SF2 RS and GST-SC35 RS into the nucleus (Fig. 5). Thus, TRN-SR is a nuclear import receptor for ASF/SF2, SC35, and likely for other RS domain-containing proteins.

**TRN-SR Binds to Several SR Proteins**

Mammalian cells contain several SR proteins in addition to ASF/SF2 (Zahler et al., 1992; Fu, 1995). Since TRN-SR binds the RS domains of both ASF/SF2 and SC35 (Fig. 4), we examined whether it can also bind other SR proteins. The SR protein fraction was purified from HeLa nuclear extracts (Zahler et al., 1992), resolved by SDS-PAGE and immobilized on a nitrocellulose membrane. By Western blotting with the anti-RS domain antibody mAb104, these purified SR proteins show the typical pattern reported previously (Fig. 6; Zahler et al., 1992). The capacity of TRN-SR to bind these proteins was determined by far Western blotting using 35S-labeled TRN-SR produced in rabbit reticulocyte lysate (Siomi et al., 1997). TRN-SR bound several of these proteins, whereas TRN1 did not.

**Figure 4.** TRN-SR binds to RS domains specifically and directly. (A) Purified GST, GST-M9, GST-IBB, GST-ASF/SF2 RS, and GST-SC35 RS were immobilized on glutathione beads and incubated with in vitro translated 35S-labeled TRN-SR (translated TRN-SR). To the reactions in the lanes marked RanQ69L, 2 μM of His-tagged RanQ69L (GTP form) was added. After binding, beads were washed with buffer containing 400 mM NaCl. Bound proteins were eluted with SDS-containing sample buffer, resolved by SDS-PAGE, and detected by Western blotting using an anti-T7 tag antibody. Molecular mass markers are shown at the left.

**Figure 5.** Recombinant TRN-SR can import RS domain-containing proteins in vitro. Import of GST-RS incubated with buffer, TRN-SR, or TRN-SR plus RanGDP was examined on digitonin-permeabilized HeLa cells. Import assays were carried out as detailed in Fig. 1.
The physiological function of the shuttling of SR proteins is not known. Both hnRNPs A1/2 proteins and SR proteins are associated with the same mRNA species as they are exported to the cytoplasm (A Izanovna-Ericsson, 1996; Visa et al., 1996) and it is thus possible that they both play a role in mRNA export. Nuclear export signals in the shuttling SR proteins have not been identified yet. The identification of nuclear export signals in shuttling SR proteins, if such exist, and of export receptors for them are issues of considerable interest that remain to be clarified.

We thank Dr. James Manley for the ASF/SF2 cDNA; Dr. Xian-dong Fu for SC35 cDNA; Dr. Dirk Gärlich for importin βΔN44 protein and RanQ69L expression plasmids; and Dr. Akihisa Myaeda for SR proteins. We also thank LiLi Wan for help on yeast two-hybrid library screening, and other members of our laboratory, especially Drs. Sara Nakielny, Haruhiko Siomi, LiLi Wan, and Robert Kerckhoff for critical reading and comments on the manuscript.

This work was supported by a grant from the National Institutes of Health (G. Dreyfuss), and by a long-term fellowship from Human Frontier Science Program Organization (N. Kataoka). G. Dreyfuss is an Investigator of the Howard Hughes Medical Institute.

Received for publication 16 February 1999 and in revised form 5 May 1999.

References

A dam, S.A., R.S. Marr, and L. Gerace. 1990. Nuclear protein import in perme-

Discussion

In this report we have identified a novel receptor, TRN-SR, as a nuclear import receptor for SR proteins. Of the known proteins currently present in the sequence databases, we note the considerable amino acid sequence homology of TRN-SR with the S. cerevisiae Mtr10p (Fig. 3). Mtr10p has been shown to be a nuclear import receptor for the yeast pre-mRNA/mRNA-binding protein Npl3 (Pemberton et al., 1997; Senger et al., 1998). Npl3p, which is also referred to as Nop3p and Nab1p, is an hnRNp protein that contains within its carboxyl terminus an RGG-box within which are several serine-arginine (SR) dipeptides (Bossie et al., 1992; Russell and Tollervey, 1992; Wilson et al., 1994; Siebel and Guthrie, 1996; Pemberton et al., 1997). The NLS of Npl3p has not been precisely delineated but is contained in this region of the protein (Senger et al., 1998). The SR dipeptides of Npl3p may be important for Mtr10p recognition, although this has not been determined. Two additional proteins, one in C. elegans and one in S. pombe, show similarity to TRN-SR (Fig. 3). Several candidate SR proteins are found in the C. elegans database, and one SR protein has been recently cloned from S. pombe (Gross et al., 1998). Therefore, these TRN-SR homologues may be the import receptors of SR proteins in these organisms.

TRN-SR binds to the RS domain of A SF/SF2 and of SC35, and these interactions are disrupted by RanQ69L (Fig. 4 A). Furthermore, TRN-SR also binds other proteins enriched in an SR protein fraction (Fig. 6). These results strongly suggest that TRN-SR is a general nuclear import receptor for SR proteins. However, we note that no binding of TRN-SR to SRp75 was detected by far Western blotting, although this protein is abundant in the fraction we tested (Fig. 6). The reason for this is unknown, but it is possible that SRp75 may have a different receptor. There are additional SR proteins, including pre-mRNA splicing factors such as 9G8, U170K, U2A F35, and 65 (Fu, 1995), as well as two large SR proteins (Bienewall et al., 1998), and it remains to be determined whether TRN-SR also mediates the nuclear import of these proteins.

Several abundant hnRNPs, including hnRNPs A1, A2, and F, are imported by TRN1 (Pollard et al., 1996; Smolen et al., 1997). Thus, in mammalian cells there are at least two nuclear import pathways for pre-mRNA/mRNA-binding proteins, one mediated by TRN1 and one by TRN-SR. The relative amounts of hnRNPs and SR proteins are important for alternative pre-mRNA splicing. For example, the ratio between hnRNPs A1 and A SF/SF2 affects 5′ splice site selection (Miyayada and Rainer, 1992; Zahler et al., 1993; Cáceres et al., 1994; Yang et al., 1994). A both of these proteins shutte between the nucleus and the cytoplasm (Pifil-Roma and Dreyfuss, 1992; A Izanovna-Ericsson et al., 1996; Cáceres et al., 1998), it is conceivable that their relative amounts in the nucleus may be controlled by regulating their rates of nuclear import. Thus, by modifying either the transportins themselves or the respective NLSs, M9 and RS, splice site selection could be modulated. Indeed, several protein kinases have been reported to phosphorylate serine residues in the RS domains of SR proteins (Gui et al., 1994a; Colwill et al., 1996; Rossi et al., 1996; Kuroyanagi et al., 1998; Okamoto et al., 1998; Wang et al., 1998). While overexpression of some of these SR protein kinases causes disruption of nuclear speckles (Gui et al., 1994a; Kuroyanagi et al., 1998; Wang et al., 1998), they do not disrupt the nuclear localization of SR proteins. However, overexpression of one SR protein kinase, Cik/Sty kinase, does cause cytoplasmic accumulation of A SF/SF2 in HeLa cells (Cáceres et al., 1998). More recently it was reported that overexpression of kinase-inactive mutant of SR protein kinase-2 causes cytoplasmic accumulation of A SF/SF2 (Kozumi et al., 1999). It will be interesting to determine the effect of RS domain phosphorylation on the SR proteins—TRN-SR interaction.

In addition to proteins of ~33 kD, that likely correspond to A SF/SF2 and SC35, proteins of ~20, 46, and 55 kD also bound specifically to TRN-SR. This observed prolineated but is contained in this region of the protein (Sen-

Figure 6. TRN-SR binds to several SR proteins. 10 μg of purified SR proteins was resolved by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was incubated with either in vitro translated 35S-labeled TRN-SR or TRN1, washed, and exposed to an x-ray film for autoradiography.
abrilized mammalian cells requires soluble cytoplasmic factors. J. Cell Biol. 119:307–316.

Izahara-Ericsson, A.T., X. Sun, N. Vasa, E. Kiseleva, T. Wurtz, and B. Danenholt. 1996. A protein of the SR family of splicing factors binds extensively to exonic Balbiani ring pre-mRNA and accompanies the RNA from the gene to the nucleus. Genes Dev. 10:2825–2833.

Arts, G. J., M. Fornerod, and I.W. Mattaj. 1998. Identification of a nuclear export receptor for TRNA. Curr. Biol. 8:305–314.

Blencowe, B. J., R. Isner, J.A. Nickerson, and P.A. Sharp. 1998. A coactivator of transcription that binds the export receptor CRM1. EMBO (Eur. Mol. Biol. Organ.) J. 17:559–569.

Bonifaci, N. M., C. Delattre, G. Barcelo, and P. Silver. 1992. A mutant nuclear protein with similarity to RNA binding proteins interferes with nuclear import in yeast. Mol. Cell. Biol. 3:875–879.

Cáceres, J. F., S. Stamm, D. M. Helfman, and A. R. Krainer. 1994. Regulation of alternative splicing in vitro by overexpression of antagonistic splicing factors. Science. 265:1700–1709.

Cáceres, J. F., T. M. Istel, G. R. Scroeton, D. L. Spector, and A. R. Krainer. 1997. Role of the modular domains of SR proteins in subnuclear localization and alternative splicing specificity. J. Cell Biol. 138:225–234.

Cáceres, J. F., G. R. Scroeton, and A. R. Krainer. 1998. A specific subset of SR proteins shuttles continuously between the nucleus and the cytoplasm. Genes Dev. 12:55–66.

Chi, N. C., E. J. Adam, and S.A. Dam. 1995. Sequence and characterization of cytoplasmic nuclear protein import factor p97. J. Cell Biol. 130:265–274.

Chi, N. C., E. J. Adam, and S. A. Dam. 1996. RanBP1 stabilizes the interaction of Ran with p97 nuclear protein import factor. J. Cell Biol. 135:5559–5569.

Colowick, C., T. Paoison, B. A. Andrews, J. Prasad, J. L. Manley, J. C. Bell, and P. D. J. 1996. The Cdk/Sty protein kinase phosphorylates SR splicing factors and regulates their intranuclear distribution. EMBO (Eur. Mol. Biol. Organ.) J. 15:2659–2667.

Dahlberg, J. E., and E. Lund. 1998. Functions of the GTPase Ran in RNA export from the nucleus. Curr. Opin. Cell Biol. 10:400–408.

Doye, V., and E. Hurt. 1997. From nucleoporins to nuclear pore complexes. Curr. Opin. Cell Biol. 9:401–411.

Forbes, D. J. 1992. Structure and function of the nuclear pore complex. Annu. Rev. Cell Biol. 8:495–527.

Fornerod, M., M. Ohno, M. Yoshida, and I.W. Mattaj. 1997. CRM 1 is an export receptor for leucine-rich nuclear export signals. Cell. 92:1051–1060.

Fridell, R. A., R. Truant, T. Thorne, R. E. Benson, and S. R. Kellen. 1997. Nuclear import of hnrNPA1 is mediated by a novel cellular cofactor related to karyopherin-beta. J. Cell Sci. 110:1325–1331.

Fu, X.-D. 1995. The superfamily of arginine- and serine-rich splicing factors. RNA. 1:563–569.

Fu, X.-D., and T. Maniatis. 1990. Factor required for mammalian spliceosome assembly is localized to discrete regions in the nucleus. Nature. 343:437–441.

Fu, X.-D., and T. Maniatis. 1992. Isolation of a complementary DNA that encodes the mammalian splicing factor SC35. Science. 256:635–638.

Fu, X.-D., A. Mayeda, T. Maniatis, and A. R. Krainer. 1992. General splicing factors SF2 and SC35 have equivalent activities in vitro, and both affect alternative 5’ and 3’ splice site selection. Proc. Natl. Acad. Sci. USA. 89:11224–11228.

Ge, H., and J. L. Manley. 1990. A protein factor, SF, controls cell-specific alternative splicing of SV40 early pre-mRNA in vitro. Cell. 62:25–34.

Ge, H., and J. L. Manley. 1991. Protein structure of the human splicing factor SF reveals similarities with Drosophila regulators. Cell. 66:373–382.

Görlich, D., S. Prehn, R. A. Laskey, and E. Hartmann. 1994. Isolation of a protein that is essential for the first step of nuclear protein import. Cell. 79:767–778.

Görlich, D., S. Kostka, R. Kraft, C. Dingwall, R. A. Laskey, E. Hartmann, S. Prehn, and E. Izaurralde. 1997. A novel class of RanGTP binding proteins. J. Cell Biol. 135:559–569.

Görlich, D., M. Dabrowski, F.R. Bischoff, U. Kutay, P. Schwarzmaier, E. Hartmann, and D. Görlich. 1998. Identification of a tRNA-specific nuclear protein import factor. Mol. Cell. 1:359–369.

Lamond, A. J., and W. C. Earnshaw. 1998. Structure and function in the nucleus. Science. 280:547–553.

Li, H., and P. M. Bingham. 1991. A arginine-serine-rich domain of the sn(u) and tra RNA processing regulators targets proteins to a subnuclear compartment implicated in splicing. Cell. 67:335–342.

Mayeda, A., and A. R. Krainer. 1992. Regulation of alternative pre-mRNA splicing by hnrNP A1 and splicing factor SF2. Cell. 68:365–375.

Istel, T., and D. L. Spector. 1998. The cellular organization of gene expression. Curr. Opin. Cell Biol. 10:323–331.

More, M. S. 1998. Ran and nuclear transport. J. Biol. Chem. 273:22857–22860.

Morioanju, J., G. Blobel, and A. R. d’au. 1995. Previously identified protein of uncertain function and karyopherin alpha1, together with karyopherin beta1 and beta2, are nuclear protein import substrates at nuclear pore complexes. Proc. Natl. Acad. Sci. USA. 92:2008–2011.

Morioanju, J., G. Blobel, and A. R. d’au. 1996a. The binding site of karyopherin alpha for karyopherin beta overlaps with a nuclear localization sequence. Proc. Natl. Acad. Sci. USA. 93:6572–6576.

Morioanju, J., G. Blobel, and A. R. d’au. 1996b. Nuclear protein import: Ran-GTP dissociates the karyopherin alpha/beta heterodimer by displacing alpha from an overlapping binding site on beta. Proc. Natl. Acad. Sci. USA. 93:7059–7062.

Nakielny, S., M. C. Siomi, H. Siomi, W. M. Michael, V. Pollard, and G. Dreyfuss. 1996. Transportin: nuclear transport receptor of a novel nuclear protein import pathway. Exp. Cell Res. 229:261–266.

Oho, M., M. Fornerod, and I.W. Mattaj. 1998. Nuclear cytoplasmic transport: the nuclear pore, 200 nanometers. Curr. Opin. Cell Biol. 10:323–336.

Okozumi, Y., H. Ono, H. Yasuda, T. Wakabayashi, Y. Nimura, and M. Hagiwara. 1998. cdcd kinase-mediated phosphorylation of splicing factor SF2/SF3. Biochem. Biophys. Res. Commun. 242:878–882.

Pemberton, L.F., G. Blobel, and J.S. Rosenblum. 1997. A distinct and parallel pathway for the nuclear import of an mRNA-binding protein. J. Cell Biol. 135:1645–1653.

Pemberton, L.F., G. Blobel, and J. S. Rosenblum. 1998. Transport routes through the nuclear pore: the importin- and arginine-rich pre-mRNA splicing factors. Proc. Natl. Acad. Sci. USA. 95:10292–10297.

Pirollo-Roma, S., and G. Dreyfuss. 1992. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. Nature. 355:730–732.

Pollard, V. W., W. M. Michael, S. Nakielny, M. C. Siomi, F. Wang, and G. Dreyfuss. 1996. A novel receptor-mediated nuclear protein import pathway. Cell.
Radu, A., G. Blobel, and M.S. Moore. 1995. Identification of a protein complex that is required for nuclear protein import and mediates docking of import substrate to distinct nucleoporins. Proc. Natl. Acad. Sci. USA. 92:1769–1773.

Rexach, M., and G. Blobel. 1995. Protein import into nuclei: association and dissociation reactions involving transport substrate, transport factors, and nucleoporins. Cell. 83:683–692.

Robbins, J., S.M. Dilworth, R.A. Laskey, and C. Dingwall. 1991. Two interdependent basic domains in nucleoplasmin nuclear targeting sequence: identification of a class of bipartite nuclear targeting sequence. Cell. 64:615–623.

Rossi, F., E. Labourier, T. Forne, G. Divita, J. Derancourt, J.F. Riou, E. Antoine, G. Cathala, C. Brunel, and J. Tazi. 1996. Specific phosphorylation of SR proteins by mammalian DNA topoisomerase I. Nature. 381:80–82.

Russell, I.D., and D. Tollervey. 1992. NOP3 is an essential yeast protein which is required for pre-rRNA processing. J. Cell Biol. 119:737–747.

Senger, B., G. Simos, F.R. Bischoff, A. Podtelejnikov, M. Mann, and E. Hurt. 1998. Mr1p10 functions as a nuclear import receptor for the mRNA-binding protein Npl3p. EMBO (Eur. Mol. Biol. Organ.) J. 17:2196–2207.

Siebel, C.W., and C. Guthrie. 1996. The essential yeast RNA binding protein Npl3p is methylated. Proc. Natl. Acad. Sci. USA. 93:13641–13646.

Singer, R.H., and M.R. Green. 1997. Compartmentalization of eukaryotic gene expression: causes and effects. Cell. 91:291–294.

Siomi, H., and G. Dreyfuss. 1995. A nuclear localization domain in the hnRNP A1 protein. J. Cell Biol. 129:551–560.

Siomi, M.C., P.S. Eder, N. Kataoka, L. Wan, Q. Liu, and G. Dreyfuss. 1997. Transportin-mediated nuclear import of heterogeneous nuclear RNP proteins. J. Cell Biol. 138:1181–1192.

Stutz, F., and M. Rosbash. 1998. Nuclear RNA export. Genes Dev. 12:3303–3319.

Visa, N., A.T. Alzhanova-Ericsson, X. Sun, E. Kiseleva, B. Bjorkroth, T. Wurtz, and B. Danenholt. 1996. A pre-mRNA-binding protein accompanies the RNA from the gene through the nuclear pores and into polysomes. Cell. 84:253–264.

Wang, H.Y., W. Lin, J.A. Dyck, J.M. Yeakley, Z. Songyang, L.C. Cantley, and X.D. Fu. 1998. SRPK 2: a differentially expressed SR protein-specific kinase involved in mediating the interaction and localization of pre-mRNA splicing factors in mammalian cells. J. Cell Biol. 140:737–750.

Weighardt, F., G. Blamonti, and S. Riva. 1995. Nucleo-cytoplasmic distribution of human hnRNP proteins: a search for the targeting domains in hnRNP A1. J. Cell Sci. 108:545–555.

Weis, K. 1998. Importins and exportins: how to get in and out of the nucleus. Trends Biochem. Sci. 23:185–189.

Weis, K., U. Ryder, and A.J. Lamond. 1996. The conserved amino-terminal domain of hSR P1 alpha is essential for nuclear protein import. EMBO (Eur. Mol. Biol. Organ.) J. 15:1818–1825.

Wilson, S.M., K.V. Datar, M.R. Paddy, J.R. Swedlow, and M.S. Swanson. 1994. Characterization of nuclear polyadenylated RNA-binding proteins in Saccharomyces cerevisiae. J. Cell Biol. 127:1173–1184.

Wozniak, R.W., M.P. Rout, and J.D. Aitchison. 1998. Karyopherins and kissing cousins. Trends Cell Biol. 8:184–188.

Yang, X., M.R. Barri, S.J. Lu, S. Rowan, Y. Ben-David, and B. Chabot. 1994. The A1 and A1B proteins of heterogeneous nuclear ribonucleoparticles modulate 5' splice site selection in vivo. Proc. Natl. Acad. Sci. USA. 91:6924–6928.

Zahler, A.M., W.S. Lane, J.A. Stolk, and M.B. Roth. 1992. SR proteins: a conserved family of pre-mRNA splicing factors. Genes Dev. 6:837–847.

Zahler, A.M., K.M. Neugebauer, W.S. Lane, and M.B. Roth. 1993. Distinct functions of SR proteins in alternative pre-mRNA splicing. Science. 260: 219–222.