REVIEW / SYNTHÈSE

Nuclear import of spliceosomal snRNPs[1]

Christiane Rollenhagen and Nelly Panté

Abstract: Uridine-rich small nuclear ribonucleoproteins (U snRNPs) are the building units of the spliceosome. These RNA and protein complexes assemble in the cytoplasm. After proper assembly and RNA maturation, mature U snRNPs are imported into the cell nucleus, where they take part in the splicing process. In this paper we review the current knowledge of how U snRNPs enter the nucleus.

Key words: U snRNPs, spliceosome, snurportin1, nuclear import, nuclear pore complex.

Résumé : Les petites ribonucléoprotéines nucléaires riches en uridine (snRNP U) sont les unités de constitution du complexe d'épissage. Ces complexes ARN-protéines s'assemblent dans le cytoplasme. Après un assemblage approprié et la maturation de l'ARN, des snRNP U matures sont transportées dans le noyau de la cellule où ils participent au processus d'épissage. Dans le présent article, nous révisons les connaissances actuelles sur la manière dont les snRNP U entrent dans le noyau.

Mots clés : snRNP U, complexe d'épissage, snurportin 1, import nucléaire, complexe du pore nucléaire.

[Traduit par la Rédaction]

Introduction

In eukaryotes, gene expression requires a series of modifications of the primary RNA transcript, called RNA processing. One aspect of RNA processing is the remarkable step of RNA splicing, in which stretches of non-coding sequences (introns) are precisely removed and coding sequences (exons) are joined together. This process is accomplished by the spliceosome, a large (~40S) RNA/protein macromolecule machine. The spliceosome is formed from several ribonucleoprotein subunits, termed uridine-rich small nuclear ribonucleoproteins (U snRNPs or "snurps"). These splicing U snRNPs are assembled in the cytosol and have to be imported into the nucleus where they function. Thus, nuclear import of U snRNPs is an important step for pre-mRNA splicing. In this paper we review recent findings on the nuclear import of U snRNPs and present our results using an electron microscopy (EM) transport assay in *Xenopus* oocyte.

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Biogenesis of U snRNP requires nuclear transport

The major building units of the spliceosome are U1, U2, U4, U5, and U6 snRNP. These spliceosomal U snRNPs are complex assemblies of RNA and proteins. Each U snRNP consists of a short (200 nucleotides) uridine-rich RNA molecule (U snRNA), a set of 7 core proteins (Sm proteins) common to all snRNPs, and several other proteins that are specific for each snRNP (Will and Lührmann 1997). The assembly of spliceosomal snRNPs is a multistep process that occurs both in the nucleus and the cytoplasm (Will and Lührmann 2001). Therefore, both nuclear import and export are involved in the biogenesis of snRNPs. As illustrated in Fig. 1, RNA components of spliceosomal snRNPs are synthesized in the nucleus and (with the exception of U6 snRNA) are exported to the cytoplasm, where they assemble into U snRNPs. Mature U snRNPs are then imported into the nucleus, where they function in the splicing process.

Spliceosomal U1, U2, U4, and U5 snRNAs are synthesized in the nucleus by RNA polymerase II and acquire a 7-methylguanosine (m^7G) cap structure at their 5′ end. This m^7G cap acts as a nuclear export signal, allowing the assembly of a nuclear cap-binding complex (CBC) composed of 2 cap-binding proteins, CBP80 and CBP20 (Izaurralde et al. 1995). Nuclear export of U snRNA is then mediated by the export receptor CRM1, which binds to CBC with the help of the PHAX adapter (Ohno et al. 2000). As with other nuclear export pathways, the small GTPase Ran controls the assembly and disassembly of the CRM1-PHAX-CBC complex; the complex is formed in the nucleus, where RanGTP is concentrated, and it is disassembled in the presence of RanGDP in the cytoplasm. The disassembly of the CRM1-PHAX-CBC

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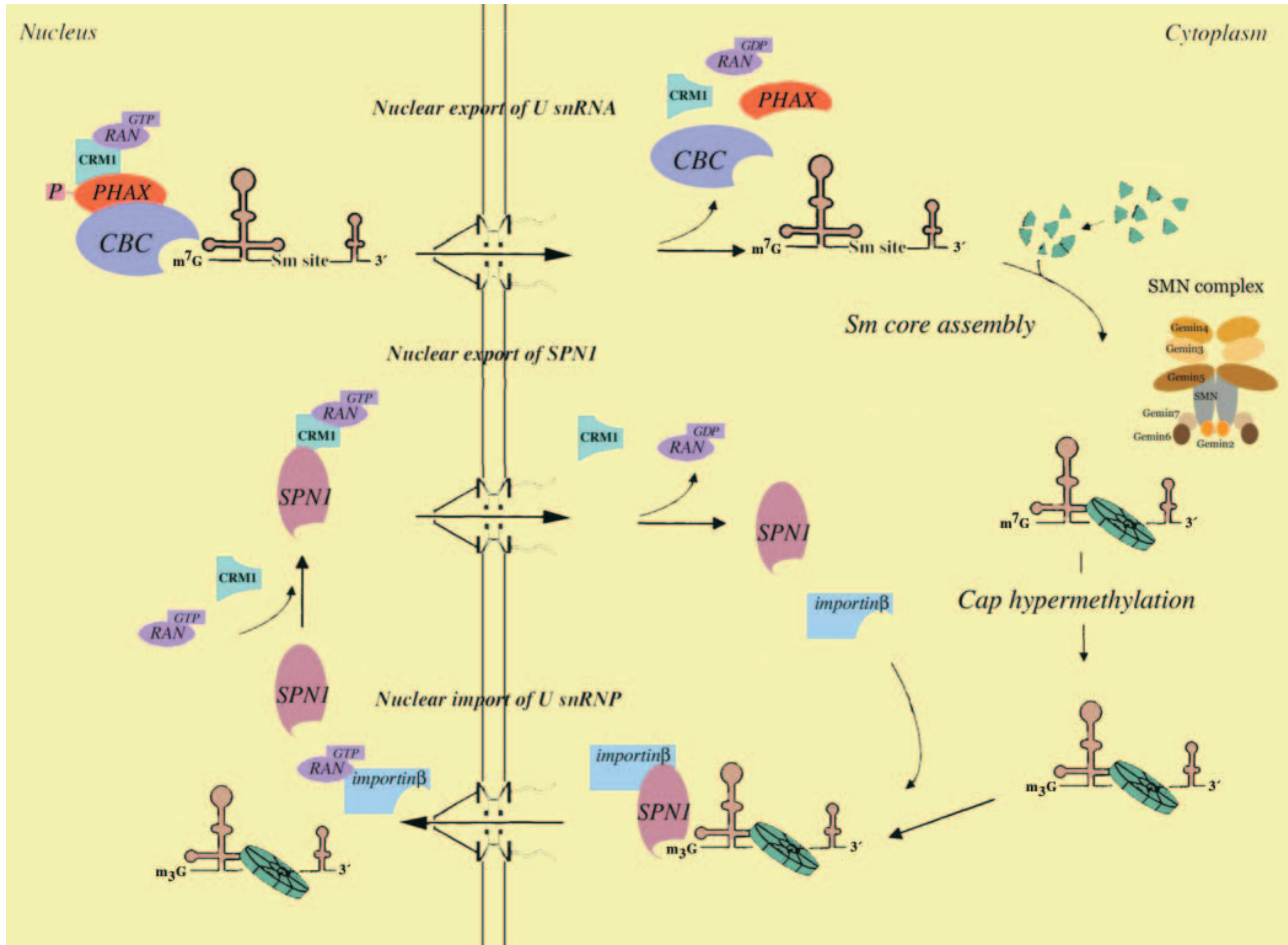
Fig. 1. Role of nuclear transport in the biogenesis of U snRNP. The formation of mature U snRNP requires nuclear export of U snRNA, assembly of the core Sm proteins in the cytoplasm, RNA processing in the cytoplasm, and nuclear import of the mature U snRNP (see text for details). Nuclear export of U snRNA is mediated by the nuclear export receptor CRM1 with the assistance of the cap-binding complex CBC and the PHAX adapter. Nuclear import of mature U snRNP is mediated by importin β with the help of the SPN1 adapter, which is also exported from the nucleus by CRM1. U snRNP can also enter the nucleus using the Sm core as a signal. For the sake of simplicity, this uncharacterized import pathway is not shown.



complex results in the release of U snRNA in the cytoplasm, where it associates with the survival of motor neurons (SMN) complex, a multiprotein complex containing the SMN protein and at least 6 other proteins called Gemins2-7 (Gubitz et al. 2004). This SMN complex directs the proper assembly of the 7 Sm proteins onto the snRNA Sm site. Because defects in SMN protein function lead to the human neuromuscular disease called spinal muscular atrophy, there has been increasing interest in the study of U snRNP assembly (Yong et al. 2004). The current model states that the SMN complex checks the U snRNAs for specific features and identifies Sm proteins. Thus, the SMN complex acts as a quality control component.

After the Sm core is assembled with the assistance of the SMN complex, the 5′ m^7G cap is hypermethylated to a trimethyl guanosine (m_3G) cap structure, and some nucleotides are removed from the 3′ end of the snRNA. These steps depend on the proper association of Sm proteins with the snRNA. Finally, mature U snRNP is imported into the nucleus using nuclear import pathways that are specific to U snRNP.

Nuclear import pathways for U snRNP

Nuclear import of molecules between 9 nm and 39 nm in diameter (Panté and Kann 2002) requires a signal residing on the imported molecule (or cargo) and soluble receptors that recognize the signal and carry the cargo through the nuclear pore complex (NPC). A major breakthrough in the study of nuclear transport has been the identification of several signals and transport receptors mediating different nuclear transport pathways (Fried and Kutay 2003). The first identified and best-studied nuclear import signal is characterized by 1 or 2 short stretches of basic amino acids called classical nuclear localization sequences (cNLSs; Dingwall et al. 1982; Kalderon et al. 1984). The receptor for the cNLS import pathway consists of 2 proteins, importin α and importin β (Görlich et al. 1994, 1995). The cNLS is recognized by importin α, which acts as an adapter protein between the cNLS-bearing protein (cNLS-protein) and importin β.

U snRNPs can enter the nucleus via 2 distinct nuclear import pathways. The first and best-characterized U snRNP nu-

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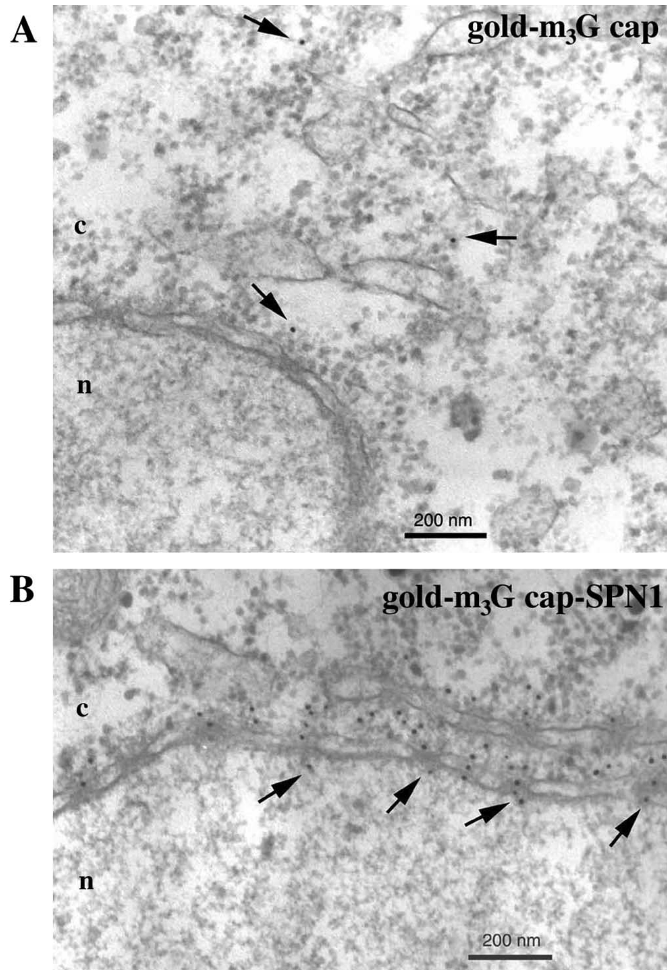
Fig. 2. Nuclear import of m_3G cap. Views of nuclear envelope cross-sections with adjacent cytoplasm (c) and nucleus (n) from *Xenopus* oocytes that have been injected with (A) m_3G cap conjugated with 8-nm gold particles (gold-m_3G cap), or (B) gold-m_3G cap that has been incubated with recombinant SPN1 (to form the gold-m_3G cap-SPN1 complex). After injection oocytes were incubated for 2 h at room temperature and processed for embedding and thin section EM as described by Rollenhagen et al. (2003). Whereas only few gold-m_3G caps interacted with the NPC and were imported into the nucleus (A), the gold-m_3G cap-SPN1 complex was targeted to the NPC and imported efficiently (B, arrows). Scale bars represent 200 nm.



clear import pathway uses the m_3G cap as the targeting signal. The second pathway's signal is the Sm core domain of the U snRNP formed by Sm proteins. Whether U snRNPs enter the nucleus using the m_3G cap pathway or the Sm core pathway depends on the type of U snRNP and the species. For example, whereas nuclear import of U1 and U2 snRNP absolutely requires an intact m_3G cap in *Xenopus* oocyte, U4 and U5 snRNP do not (Marshallsay and Lührmann 1994).

Both pathways for the U snRNP nuclear import converge in importin β, the receptor for the cNLS import pathway. However, they are different in the targeting signals and the adapters that recognize the signals and bind to importin β. The protein snurportin 1 (SPN1) has been identified as the adapter for the m_3G cap-dependent nuclear import pathway (Huber et al. 1998). However, the adapter for the Sm core-dependent nuclear import pathway remains to be identified. Nevertheless, because the SMN protein binds importin β in vitro (Narayanan et al. 2002), it has been proposed that SMN serves as an adapter between the Sm core and importin β. However, the interaction between the SMN complex and importin β has not yet been characterized. In addition, it is not ruled out that an unknown adapter protein mediating the interaction between the SMN complex and importin β exists.

In contrast to the Sm core-dependent import pathway, the m_3G cap-dependent import pathway has been characterized in some detail. The adapter SPN1 binds specifically to the m_3G cap signal and importin β to mediate the nuclear import of U snRNP. SPN1 consists of 2 domains: the C-terminus, which interacts with the m_3G cap, and the N-terminus (the importin β-binding domain or IBB), which binds importin β. Importin β then mediates the interaction with the NPC and translocates the U snRNP import complex through the NPC.

Studies with in vitro import assays using digitonin permeabilized cells have demonstrated that U snRNPs can enter the nucleus using either import pathway (Huber et al. 1998; Narayanan et al. 2004). Thus, in vitro each pathway is independent of the other. This finding is also supported by our EM studies using *Xenopus* oocytes microinjected with the m_3G cap, which has been conjugated with gold particles (gold-m_3G cap). While conducting similar studies to those by Fischer and Lührmann (1990), which used a truncated U1 RNA that contained the m_3G cap but did not bind to the Sm proteins, we found that the m_3G cap signal alone is sufficient for the nuclear import of the gold-m_3G cap (Fig. 2A). However, the efficiency of the nuclear import of the gold-m_3G cap was low, most likely because of the low availability of SPN1 in the cytosol (Huber et al. 2002). Consistent with this idea is the finding that when the gold-m_3G cap was first incubated in vitro with recombinant SPN1 and then injected into *Xenopus* oocytes, the nuclear import of the gold-m_3G cap dramatically increased (Fig. 2B). Alternatively, an explanation for the different results with the gold-m_3G cap and the gold-m_3G cap-SPN1 is that other domains of the U snRNP may function in recruiting SPN1 and (or) targeting the U snRNPs to the NPC.

One of the characteristics of importin β-mediated nuclear import is the ability of this receptor to interact with the small GTPase Ran (Floer and Blobel 1996; Lounsbury et al. 1996). It has been well established that for the cNLS import pathway the binding of nuclear RanGTP to importin β disassembles the cargo-importin β complex (Izaurralde et al. 1997). However, this seems to be different for the m_3G cap-dependent import pathway. It has recently been demonstrated by Huber et al. (2002) and confirmed by Rollenhagen et al. (2003) that the m_3G cap-dependent import pathway requires importin β but not Ran or energy (summarized in Fig. 3).

Molecular differences between the m_3G cap-dependent and the cNLS nuclear import pathways

Competition studies first suggested that both the signal and the factors mediating the U snRNP import pathway

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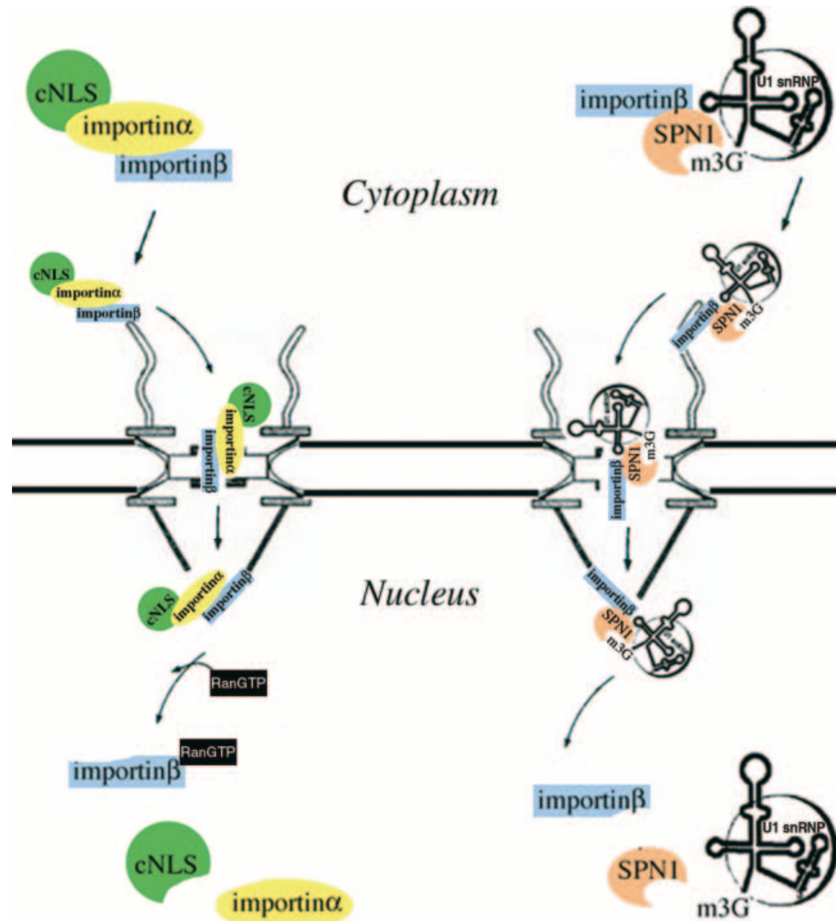
Fig. 3. Comparison of cNLS and m_3G cap-dependent nuclear import pathways. Both pathways are mediated by the nuclear pore complex (NPC)-interacting transport receptor, importin β. However, the adapter recognizing the import signal and the requirement of Ran and energy to dissociate the import complex from the NPC distinguishes each pathway.



were different from those mediating the cNLS import pathway (Fischer et al. 1991; Michaud and Goldfarb 1992). It is then very surprising that importin β mediates both import pathways (Palacios et al. 1997). Nevertheless, it has been demonstrated that even if both pathways are mediated by importin β, there are notable differences between them, which can be explained by the differences between adapter proteins that bridge the import signal to importin β (Rollenhagen et al. 2003).

The adapters for the cNLS import pathway and the m_3G cap import pathway are importin α and SPN1, respectively. Even though they both act as adapters and contain a highly similar IBB binding domain at their N-terminus (Huber et al. 1998), there are some indications that these 2 proteins are distinct. First, the C-terminal domains of these 2 proteins, which bind to the nuclear import signals are structurally distinct (Huber et al. 1998). Second, the nuclear export of SPN1 and importin α is mediated by different export receptors (Kutay et al. 1997; Paraskeva et al. 1999). Third, the affinity of SPN1 for importin β is lower than the affinity of importin α for importin β (Huber et al. 1998). Finally, SPN1 and importin α bind to different regions of importin β (Rollenhagen et al. 2003).

The final difference may explain why Ran and energy are not required for the m_3G cap-dependent nuclear import of U snRNP, whereas both are necessary for the cNLS-protein to enter the nucleus. Importin α binds to the 3/4 C-terminal region of importin β (residues 256–876) (Cingolani et al. 1999; Kutay et al. 1997). In contrast, SPN1 binds to the N-terminal 452 residues of importin β, a region that does not bind to importin α (Rollenhagen et al. 2003). Because the IBB domain of SPN1 and importin α are highly similar (Huber et al. 1998), it is very surprising to find different modes of interaction between importin β and these adapters. Although the importin β-binding domain for SPN1 has not yet been narrowed down, it partially overlaps with the Ran binding domain of importin β (residues 1–342) (Kutay et al. 1997; Vetter et al. 1999). Thus, it can be speculated that SPN1 blocks (or changes) the Ran binding site of importin β. This results in the inability of importin β to bind Ran and explains why Ran is not required for the U snRNP nuclear import but is required for the cNLS-protein nuclear import.

Consistent with the finding that the IBB domains for each adapter and the requirement for Ran are different, it has been found that the IBB domains of importin α and SPN1 interact differently with the nuclear side of the NPC (Rollenhagen et al. 2003). Using microinjection of the IBB domain labeled with gold particles into the cytoplasm of *Xenopus* oocytes followed by EM analysis, Rollenhagen et

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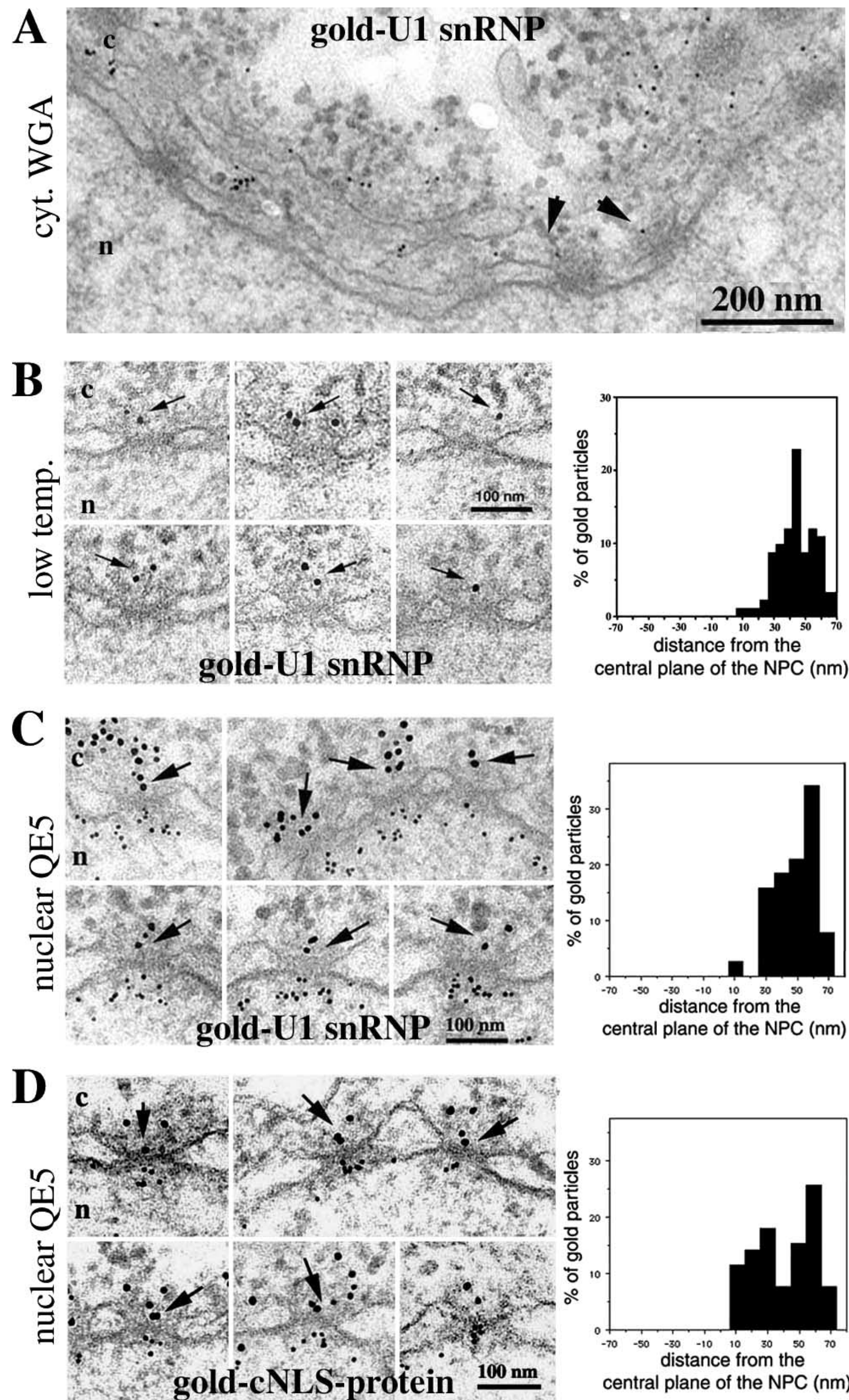
Fig. 4. Nuclear pore complex (NPC)-arrested intermediates for U1 snRNP and cNLS import pathways under different non-permissive conditions. Views of nuclear envelope cross-sections with adjacent cytoplasm (c) and nucleus (n) from *Xenopus* oocytes that have been injected with U1 snRNP conjugated with 8-nm gold particles (gold-U1 snRNP, A–C) or cNLS-protein conjugated with 8-nm gold particles (gold-cNLS-protein, D). Different inhibitory conditions were employed. (A) Oocytes were cytoplasmically injected with 50 nL of 10 mg/mL WGA. The injected oocytes were then incubated for 6 h at room temperature. After incubation the oocytes were again injected with gold-U1 snRNP. (B) Oocytes were cooled on ice for at least 30 min before microinjection and kept on ice during and after microinjection. (C, D) Oocytes were nuclear injected with 20 nL of the QE5 antibody conjugated with 6-nm gold particles. The injected oocytes were then incubated for 2 h at room temperature. After incubation the oocytes were again injected in their cytoplasm with (C) gold-U1 snRNP or (D) gold-cNLS-protein. After the last injection, the oocytes were incubated for 2 h at room temperature (A, C, D) or on ice (B) and processed for embedding and thin section EM as described by Rollenhagen et al. (2003). Arrows indicate gold particles associated with the NPC. Quantification of gold particles associated with the NPC for experiments B–D is shown on the right. For each histogram, the positions of 80–90 gold particles were plotted. Each histogram represents data from 3 different experiments. Scale bars represent 100 nm and 200 nm, as indicated.



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al. (2003) found that the IBB of SPN1 is efficiently released into the nucleus, whereas the IBB of importin α remains associated with the nuclear basket of the NPC. Because RanGTP binds importin β at the nuclear basket (Görlich et al. 1996), our explanation for the different interactions of the IBBs with the nuclear basket is that the dissociation of the cargo-receptor complex for the m_3G cap import pathway is faster than for the cNLS import pathway. This is because the former does not have to wait for RanGTP to bind to importin β to release the cargo into the nucleus.

The fact that the different Ran and energy requirements for the m_3G cap-dependent and the cNLS import pathways rely on the adapter molecule was elegantly demonstrated by Huber et al. (2002). They fused the IBB domain of importin α with a SPN1 molecule that lacked its own IBB domain and showed that the import of U snRNPs in the presence of the SPN1 molecule with swapped IBB required Ran and energy.

U snRNP and the cNLS import pathways have different NPC-arrested intermediates

Molecular differences between SPN1 and importin α also explain the level of interaction between the import complex (complex of the cargo with the adapter and importin β) and the NPC. Using microinjection of gold-labeled cargo into the cytoplasm of *Xenopus* oocytes followed by EM analysis, researches have depicted the in vivo interactions between the import complex and the NPC for both m_3G cap-dependent and cNLS import pathways (Panté and Aebi 1996; Rollenhagen et al. 2003). Both gold-labeled U1 snRNP and gold-labeled cNLS-protein are found in transit to the NPC when they are injected into *Xenopus* oocytes under permissive conditions (Panté and Aebi 1996; Rollenhagen et al. 2003). Further ultrastructural analysis of U1 snRNP and cNLS import pathways under nonpermissive conditions revealed different NPC-arrested intermediates (presented in Fig. 4 and summarized in Fig. 5).

Under permissive conditions, both complexes interact with the cytoplasmic filaments of the NPC, the cytoplasmic entrance, and the nuclear exit of the NPC central channel, as well as the nuclear basket of the NPC (summarized in Fig. 5). However, in contrast to the cNLS import pathway, the U1 snRNP import pathway has 2 NPC-binding sites at the cytoplasmic filament of the NPC (Rollenhagen et al. 2003).

Biochemical and physiological inhibitors of nuclear transport have been used to characterize NPC-arrested intermediates for both U snRNP and cNLS import pathways (Panté and Aebi 1996; Rollenhagen et al. 2003). One of these is the lectin wheat germ agglutinin (WGA), which inhibits nuclear import by interacting with the glycosylated residues of NPC proteins (nucleoporins). It has been previously shown that WGA has a limited inhibitory effect on the nuclear import of U snRNPs under conditions that completely inhibit the nuclear import of cNLS-proteins (Michaud and Goldfarb 1992; Marshallsay and Lührmann 1994). EM analysis of both import pathways in *Xenopus* oocytes revealed that the cNLS-protein and U1 snRNP import complexes have different NPC-arrested intermediates when WGA is present in the cytosol. Whereas the cNLS-import complex interacts with the cytoplasmic filaments of the NPC (Panté and Aebi 1996), U1 snRNP does not significantly bind to the NPC of *Xenopus* oocytes pre-injected with WGA in their cytoplasm (Fig. 4A). Similar results were obtained when the antibody QE5 against the nucleoporins CAN/Nup214 (located at the NPC cytoplasmic filaments; Panté et al. 1994), p62 (located at both the cytoplasmic and the nuclear side of the NPC central channel; Guan et al. 1995), and Nup153 (located at the NPC nuclear basket; Panté et al. 1994) was injected into the cytoplasm of *Xenopus* oocytes (Rollenhagen et al. 2003). Cytoplasmic QE5 blocked the binding of U1 snRNP but not the binding of cNLS-protein to the cytoplasmic filaments of the NPC (Fig. 5). From these findings we can conclude that cNLS-protein and U snRNP import pathways have different initial interactions with the NPC, and the nucleoporin CAN/Nup214 is the first NPC binding site for the U snRNP import pathway.

A second condition that yields NPC-arrested intermediates is the inhibition of nuclear import at low temperatures. Palacios et al. (1996) report the lack of U snRNP docking at the NPC at low temperatures, a condition that arrests cNLS-proteins at the NPC. Our ultrastructural analysis in *Xenopus* oocytes revealed slightly different results. As documented in Fig. 4B, we have found that at low temperatures gold-U1 snRNP is arrested at the cytoplasmic filaments of the NPC. This is in contrast to the cNLS import complex, which at low temperatures is found arrested at the cytoplasmic entrance of the NPC central channel (Panté and Aebi 1996; Fig. 5). Similar results were observed when the antibody QE5 was injected into the nucleus of *Xenopus* oocytes to block nucleoporins Nup153 and p62. As documented in Fig. 4C and 4D, whereas gold-U1 snRNP was found arrested at the cytoplasmic filaments of the NPC in the presence of nuclear QE5, gold-cNLS-protein was arrested at the NPC central channel under the same conditions. Thus, these inhibitory conditions block the release of the import complex from the NPC cytoplasmic filaments for the U snRNP import pathway but not for the cNLS import pathway.

In summary, 4 different inhibitory conditions (cytoplasmic WGA, cytoplasmic QE5, low temperatures, and nuclear QE5) have illustrated that U snRNP and cNLS import pathways have different NPC-arrested intermediates (Fig. 5). In addition, the data strongly suggest that the import complex route through the NPC is specific for each nuclear import pathway.

SPN1 mediates the initial binding of the U snRNP import complex to the NPC

Because importin β mediates the interaction between the import complex and the NPC, it is very surprising to find different NPC-arrested intermediates for U snRNP and cNLS import pathways. Two possible explanations for these data are that since the adapters bind to different domains of importin β, NPC-arrested intermediates involve different interactions of the NPC with different parts of importin β, and (or) that importin β is not directly involved in some NPC interactions.

The latter explanation seems to be valid for the initial interaction of the U snRNP import complex with the NPC. Because antibody QE5 against nucleoporin CAN/Nup214

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Fig. 5. Summary of NPC-arrested intermediates for the cNLS and the U snRNP nuclear import pathways under different conditions. (A) Schematic diagram of the NPC indicating some NPC components. (B) Schematic diagram of NPCs, with black dots representing sites where gold particles were found in experiments using microinjection of gold-labeled cargo (gold-labeled U1 snRNP and gold-labeled cNLS-protein) into the cytoplasm of *Xenopus* oocytes followed by EM analysis.

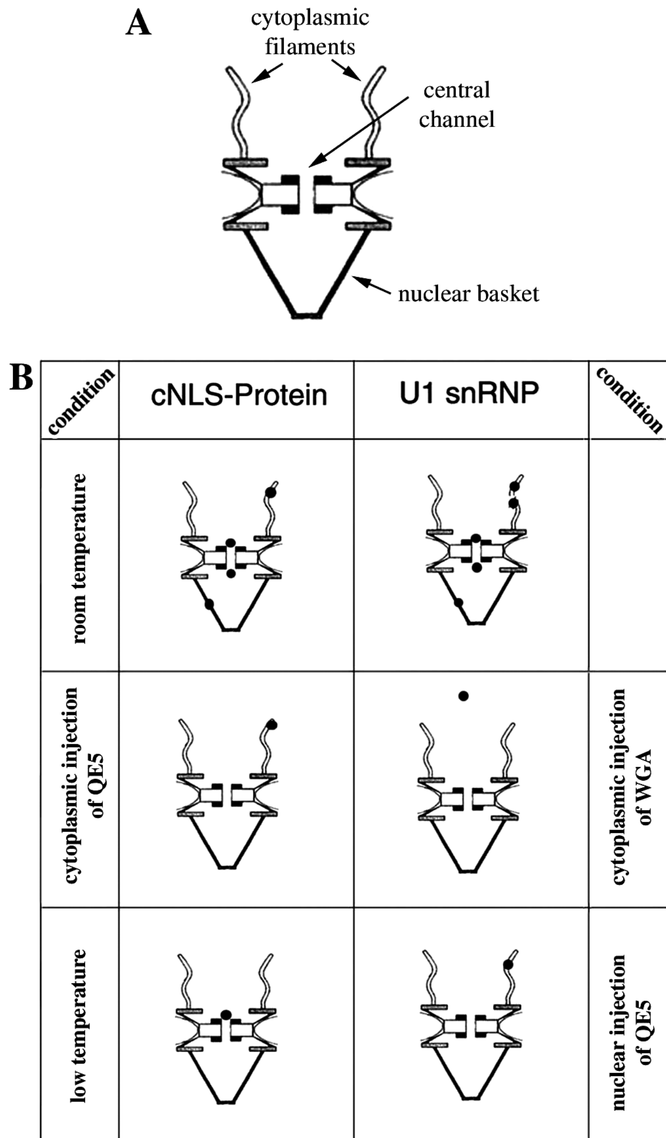
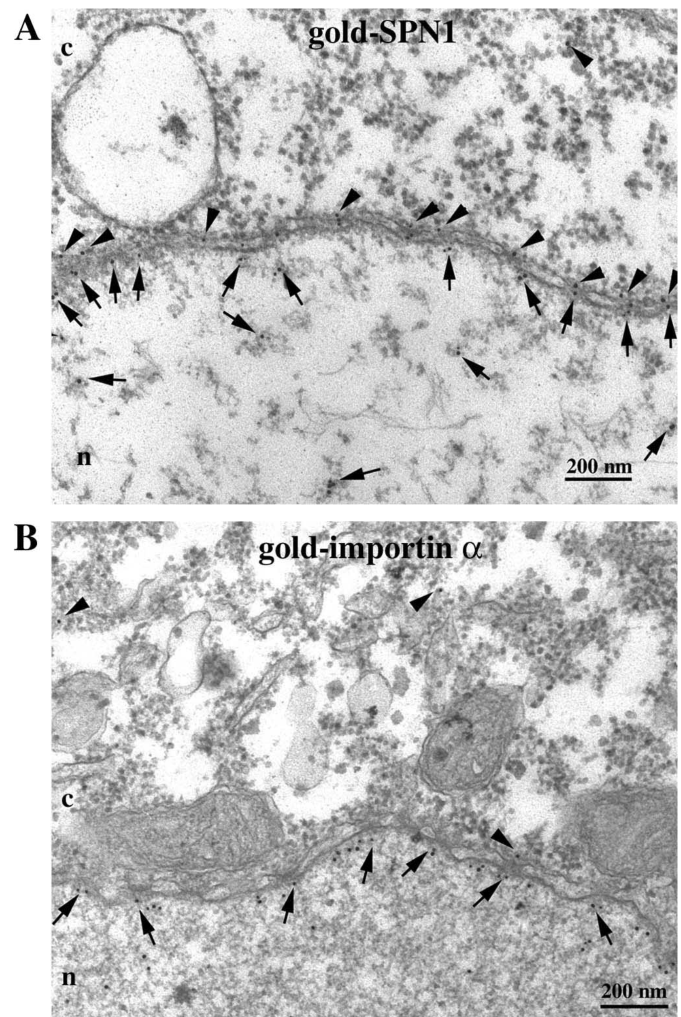


Fig. 6. Nuclear export of SPN1 and importin α. Views of nuclear envelope cross-sections with adjacent cytoplasm (c) and nucleus (n) from *Xenopus* oocytes that have been injected into their nucleus with (A) SPN1 conjugated with 8-nm gold particles (gold-SPN1) or (B) importin α conjugated with 8-nm gold particles (gold-importin α). After injection, oocytes were incubated for 1 h at room temperature and processed for embedding and thin section EM as described by Rollenhagen et al. (2003). Importin α is rapidly released from the cytoplasmic side of the NPC, whereas SPN1 remains associated with the nuclear pore complex. Scale bars represent 200 nm.



(located at NPC cytoplasmic filaments) blocks the binding of U snRNP to NPC cytoplasmic filaments, it follows that this nucleoporin is involved in the initial docking of U snRNP to the NPC. To demonstrate this, Rollenhagen et al. (2003) performed pull-down experiments with immobilized SPN1 and importin α. As expected, CAN/Nup214 was revealed in the pull-down assays performed with SPN1 but not in similar pull-down experiments with importin α. Thus, SPN1 is needed for the initial interaction of U snRNP and the NPC. However, SPN1 does not interact with CAN/Nup214 directly, but via CRM1, the nuclear export receptor of SPN1 that binds directly to CAN/Nup214 (Fornerod et al. 1997). This result points to another difference between U snRNP and cNLS import pathways: whereas the cNLS-protein binds its adapter in the cytoplasm (Görlich et al. 1995), U snRNP binds its adapter at the NPC. This is because SPN1 is concentrated at the NPC (Huber et al. 2002), whereas importin α is located in the cytosol (Görlich et al. 1994). Consistent with this explanation, when gold-conjugated SPN1 is injected into the nucleus of *Xenopus* oocytes, the gold particles are exported through the NPC but remain associated with NPC cytoplasmic filaments (Fig. 6A). This is in contrast to importin α, which is rapidly exported from the nucleus and released in the cytosol (Fig. 6B). Thus, SPN1 seems to function like an NPC-located transport factor.

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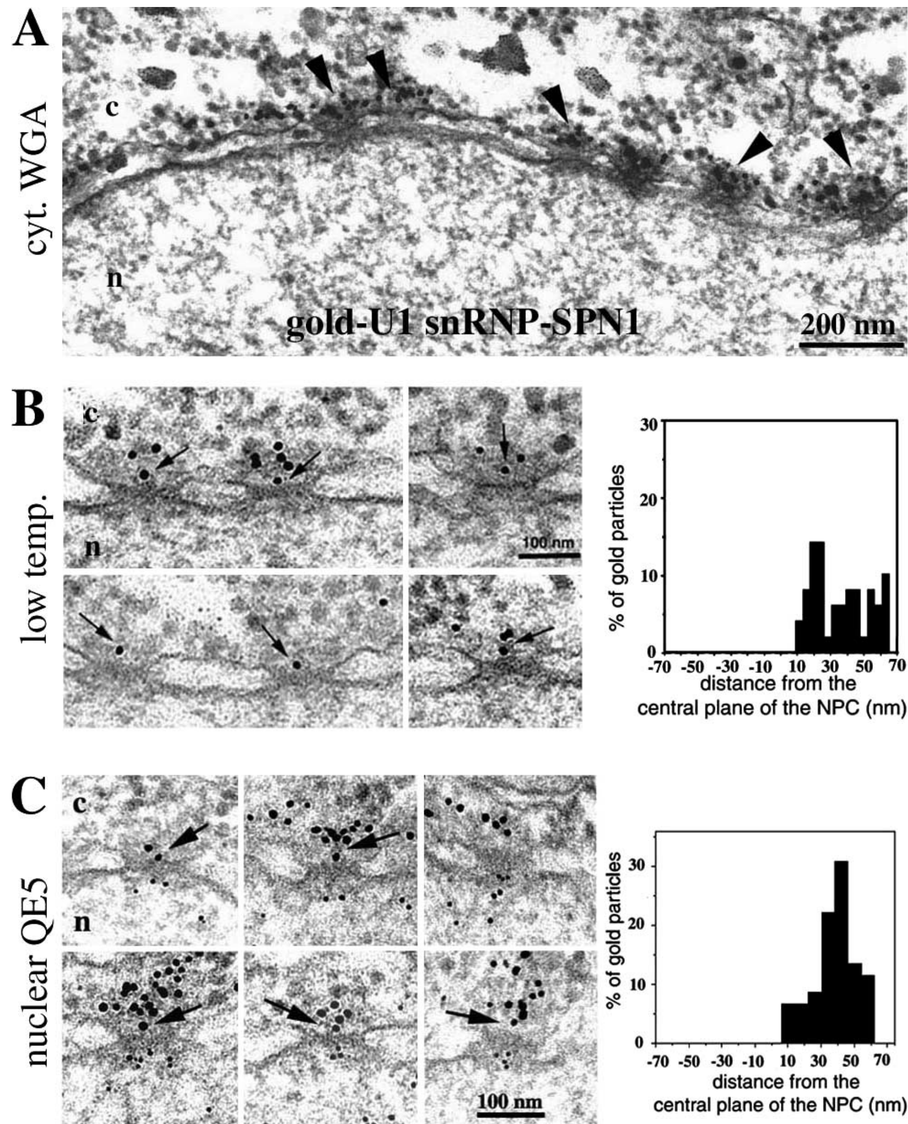
Fig. 7. Nuclear pore complex (NPC)-arrested intermediates for the U1 snRNP-SPN1 complex. Views of nuclear envelope cross-sections with adjacent cytoplasm (c) and nucleus (n) from *Xenopus* oocytes that have been injected with the complex gold-U1 snRNP-SPN1. This complex was formed by mixing recombinant SPN1 with gold-labeled U1 snRNPs in a 1:1 molar ratio followed by incubation for 20 min at room temperature. Different inhibitory conditions were employed (indicated on the left), and experiments were performed as indicated in the legend to Fig. 4. However, oocytes were injected with gold-U1 snRNP-SPN1. Arrows and arrowheads indicate gold particles associated with the NPC. Quantification of gold particles associated with the NPC for experiments B and C is shown on the right. For each histogram, the positions of 80–90 gold particles were plotted. Each histogram represents data from 3 different experiments. Scale bars represent 100 nm and 200 nm, as indicated.



The U snRNP import pathway has 2 binding sites at NPC-cytoplasmic filaments

Because the first NPC-binding site for the U snRNP import pathway is mediated by SPN1 and since many inhibitory conditions block the release of the U snRNP import complex from the NPC, Rollenhagen et al. (2003) performed experiments injecting *Xenopus* oocytes with the in vitro-formed gold-U1 snRNP-SPN1 complex. Under permissive conditions, this complex was efficiently imported into the nucleus of *Xenopus* oocytes (Rollenhagen et al. 2003). However, whereas the NPC of oocytes injected with gold-U1 snRNP revealed most of the gold particles at NPC cytoplasmic filaments, the NPC of oocytes injected with the gold-U1 snRNP-SPN1 complex yielded gold particles in transit through the NPC and at other NPC binding sites. Thus, the release of the import complex from NPC cytoplasmic filaments seems to be a rate-limiting event.

Rollenhagen et al. (2003) also performed experiments in which oocytes were first injected with antibody QE5 (to block nucleoporin CAN/Nup214) and then with the gold-U1 snRNP-SPN1 complex. Very surprisingly, it was found that,

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in contrast to gold-U1 snRNP, this complex was bound to NPC cytoplasmic filaments. The data indicate that when SPN1 is present in the import complex, it can skip the first NPC binding site at CAN/Nup214. Thus, the U snRNP import pathway has 2 binding sites at NPC-cytoplasmic filaments: at CAN/Nup214, where the cargo binds to SPN1, and one that requires the import complex to be formed first.

Cytoplasmic NPC-arrested intermediates for U snRNP and cNLS pathways are similar when U snRNP is in a preformed complex with SPN1

Similar to the experiments performed by Rollenhagen et al. (2003) with the in-vitro formed gold-U1 snRNP-SPN1 complex and the QE5 antibody, we have performed experiments with this complex using other inhibitory conditions. These were the same 3 nonpermissive conditions (cytoplasmic WGA, low temperatures, and nuclear QE5) that we have used for the experiments with gold-U1 snRNP (Fig. 4). As documented in Fig. 7, we have found that the gold-U1 snRNP-SPN1 complex yields NPC-arrested intermediates that are different from those obtained with the gold-U snRNP but are the same as the following intermediates obtained with the gold-cNLS-protein: (*i*) cytoplasmic preinjection of WGA inhibited the binding of U1 snRNP to the NPC (Fig. 4A) but, similar to the cNLS-protein, led to an arrest of the U1 snRNP-SPN1 complex to NPC cytoplasmic filaments (Fig. 7A); (*ii*) low temperatures arrested the U1 snRNP at the cytoplasmic filaments of the NPC (Fig. 4B), but the U1 snRNP-SPN1 complex (Fig. 7B) and cNLS-protein (Panté and Aebi 1996) at the cytoplasmic entrance of the NPC central channel; and (*iii*) nuclear injections of QE5 arrested the U1 snRNP at the cytoplasmic filaments of the NPC (Fig. 4C), but the U1 snRNP-SPN1 complex (Fig. 7C) and cNLS-protein (Fig. 4D) at the cytoplasmic entrance of the NPC central channel.

The NPC-arrested intermediates found for U1 snRNP, U1 snRNP-SPN1 complex, and cNLS-protein are consistent with the notion that SPN1, and not importin α, exists in very low concentrations in the cytosol. Thus, if SPN1 is artificially provided to the cell, the NPC-arrested intermediates of U snRNP and cNLS pathways are the same. In addition, because the nonpermissive conditions used in our experiments inhibited nuclear export, the different NPC-arrested intermediates for U snRNP and U1 snRNP-SPN1 may be the result of blocking the nuclear export of SPN1. Thus, some of the differences between the 2 importin β-mediated nuclear import pathways can be explained by the differences between their adapter proteins.

The route of the U snRNP nuclear import complex through the NPC

The data reported by Rollenhagen et al. (2003) and presented in this review indicate that, similar to nuclear import of cNLS-proteins, nuclear import of U snRNP involves several steps at the molecular level. For the m_3G cap-dependent nuclear import of U snRNP, the first step involves the recognition of the m_3G cap by SPN1. This association takes place at the nucleoporin CAN/Nup214, located at NPC cytoplasmic filaments. CAN/Nup214 is involved in CRM1-mediated nuclear export and bridges SPN1 to CAN/Nup214 (Fornerod et al. 1997). Subsequently, SPN1 binds to importin β to form the import complex. The exact step in which importin β is incorporated into the complex is not yet clear. It could be at CAN/Nup214, immediately after the cargo-SPN1 complex is released from CAN/Nup214, or at other NPC sites. Next, the import complex (with or without importin β) binds to a second site at the NPC cytoplasmic filament. From this site, the U snRNP import complex is then delivered to the cytoplasmic entrance of the NPC central channel and is translocated to the nuclear side of the NPC. At the nuclear side of the NPC, the U snRNP import complex interacts with the nuclear basket. At the nuclear basket, the U snRNP is dissociated from SPN1 and importin β, and it is released into the nucleus. This last step does not require interactions between nuclear RanGTP and importin β.

Conclusions

The splicing of pre-mRNA is a fundamental step on eukaryotic gene expression. Cells have developed a multi-step mechanism to ensure that only properly assembled U snRNPs reach the nucleus to participate in splicing. This mechanism involves the nuclear import of U snRNPs, which is mediated by 2 import signals. These signals are formed after the assembly of U snRNPs in the cytoplasm, and they lead to 2 nuclear import pathways for U snRNPs. Although progress has been made in characterizing the U snRNP import pathway mediated by the m_3G cap signal, very little is known about the U snRNP import pathway mediated by the Sm core signal. Moreover, it is still not fully understood how these 2 import pathways interact with each other in vivo. For example, it is unknown whether they function simultaneously or sequentially, or whether the 2 are redundant. An issue that has not yet been addressed is the effect of nuclear import defects on the mechanism and the efficiency of the splicing process. Thus, despite of the progress on nuclear import of U snRNP reviewed in this paper, many aspects of this process remain poorly understood.

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