Identification and characterization of *Drosophila* Snurportin reveals a role for the import receptor Moleskin/importin-7 in snRNP biogenesis

Amanda Hicks Natalizio[a] and A. Gregory Matera[a,b,c,d,e]
[a]Curriculum in Genetics and Molecular Biology, Departments of [b]Biology and [c]Genetics, [d]Program in Molecular Biology and Biotechnology, and [e]Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC 27599

ABSTRACT **Nuclear import is an essential step in small nuclear ribonucleoprotein (snRNP) biogenesis. Snurportin1 (SPN1), the import adaptor, binds to trimethylguanosine (TMG) caps on spliceosomal small nuclear RNAs. Previous studies indicated that vertebrate snRNP import requires importin-β, the transport receptor that binds directly to SPN1. We identify CG42303/*snup* as the *Drosophila* orthologue of human *snurportin1* (*SNUPN*). Of interest, the importin-β binding (IBB) domain of SPN1, which is essential for TMG cap–mediated snRNP import in humans, is not well conserved in flies. Consistent with its lack of an IBB domain, we find that *Drosophila* SNUP (dSNUP) does not interact with Ketel/importin-β. Fruit fly snRNPs also fail to bind Ketel; however, the importin-7 orthologue Moleskin (Msk) physically associates with both dSNUP and spliceosomal snRNPs and localizes to nuclear Cajal bodies. Strikingly, we find that *msk*-null mutants are depleted of the snRNP assembly factor, survival motor neuron, and the Cajal body marker, coilin. Consistent with a loss of snRNP import function, long-lived *msk* larvae show an accumulation of TMG cap signal in the cytoplasm. These data indicate that Ketel/importin-β does not play a significant role in *Drosophila* snRNP import and demonstrate a crucial function for Msk in snRNP biogenesis.**

Monitoring Editor
Karsten Weis
University of California, Berkeley

Received: Mar 1, 2013
Revised: Jul 15, 2013
Accepted: Jul 15, 2013

INTRODUCTION

Biogenesis of uridine-rich small nuclear ribonucleoproteins (U snRNPs) is biphasic, taking place in two distinct cellular subcompartments (reviewed in Matera *et al.*, 2007). Small nuclear RNAs (snRNAs) of the Sm class are transcribed by a specialized form of RNA polymerase II (Hernandez and Weiner, 1986) and then exported to the cytoplasm for assembly into pre-snRNPs by the export adaptor, PHAX (Ohno *et al.*, 2000). Once in the cytoplasm, the survival motor neuron (SMN) complex mediates the assembly of the Sm core RNP by loading seven Sm proteins onto the snRNA (Meister *et al.*, 2002; Pellizzoni *et al.*, 2002).

After Sm core assembly, the 5′-end methylguanosine cap structure of the snRNA is hypermethylated to form a trimethylguanosine (TMG) cap by the RNA methyltransferase (Tgs1; Mouaikel *et al.*, 2002), and this modification is believed to be a signal for nuclear import (Mattaj and De Robertis, 1985; Hamm *et al.*, 1990; Fischer *et al.*, 1993; Palacios *et al.*, 1997). The partially assembled snRNPs are then transported back into the nucleus via the import adaptor, snurportin1 (SPN1) and the import receptor, importin-β (Impβ; Palacios *et al.*, 1997; Huber *et al.*, 1998). SPN1 contains two coplanar β-sheets linked by two crossing β-strands (Strasser *et al.*, 2005) that selectively bind the TMG cap. Once in the nucleus, snRNPs undergo additional maturation steps within the nucleoplasm and/or in Cajal bodies (Jády *et al.*, 2003). RNP import is a crucial step in the biogenesis of snRNPs, as these factors cannot participate in active splicing without proper import into the nucleus.

This article was published online ahead of print in MBoC in Press (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E13-03-0118) on July 24, 2013.

Address correspondence to: A. Gregory Matera (matera@unc.edu).

Abbreviations used: DAPI, 4′,6-diamidino-2-phenylindole; dSmB, *Drosophila* Smith B antigen; dSMN, *Drosophila* survival motor neuron; dSNUP, *Drosophila* snurportin; dsRNA, double-stranded RNA; GFP, green fluorescent protein; IBB, Importin-β binding domain; Imp7, Importin-7; Impβ, Importin-β; IP, immunoprecipitation; Msk, Moleskin; PHAX, phosphorylated adaptor for snRNA export; RNAi, RNA interference; RNP, ribonucleoprotein; RT-PCR, reverse transcription-PCR; SMN, survival motor neuron; snRNP, small nuclear RNP; SNUPN, human Snurportin; SPN1, Snurportin1; TMG, 2,2,7-trimethylguanosine; U snRNA, uridine-rich small nuclear RNA; VFP, Venus fluorescent protein; WT, wild type; Xpo1/Crm1, Exportin1.

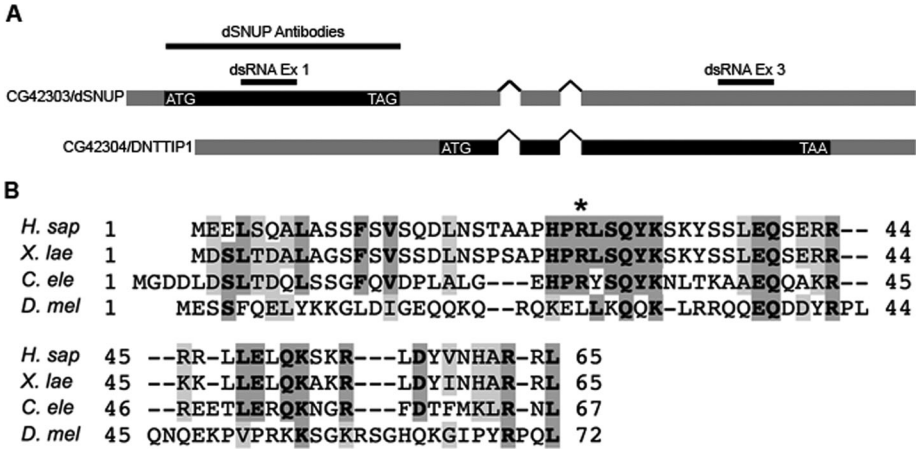


FIGURE 1: CG42303 is the *Drosophila* Snurportin orthologue. (A) Cartoon of bicistronic transcripts predicted from dSNUP/DNTTIP1 locus. Translated regions are shown in black and untranslated regions in gray. Black bars indicate regions targeted by dsRNAs or dSPN antibodies. (B) Alignment of N-termini SPN orthologues. The IBB of hSPN is defined as amino acid residues 26–65, based on similarity with the IBB of importin-α (Huber *et al.*, 1998). *Homo sapiens*, *Xenopus laevis*, *C. elegans*, and *D. melanogaster* SPN proteins are aligned, with identities in dark gray and similarities in light gray. Asterisk indicates human residue R27, which abolishes importin-β binding when mutated (Ospina *et al.*, 2005).

U snRNPs do not contain a classical nuclear localization signal. Instead, U snRNP import depends on two noncanonical signals: the TMG cap and the Sm core (Fischer *et al.*, 1993; Marshallsay and Lührmann, 1994). SPN1 is the import adaptor for the TMG cap pathway (Huber *et al.*, 1998), whereas the SMN complex (or some component thereof) is believed to function as the import adaptor for the Sm core pathway (Narayanan *et al.*, 2004). Thus bipartite import signals are believed to ensure that only functional RNPs are imported into the nucleus.

U snRNP import is complicated by the fact that individual U snRNPs have distinct import requirements. Although the TMG cap is required for U1 and U2 snRNP import in frog oocytes, it is not required in somatic cells or for U4 and U5 snRNPs in oocytes (Fischer *et al.*, 1991, 1993; Wersig *et al.*, 1992). The observed TMG cap dependence of snRNP import is cell-type specific rather than species specific (Fischer *et al.*, 1994). In digitonin-permeabilized human cells, recombinant SPN and Impβ are necessary and sufficient for U1 snRNP import (Huber *et al.*, 2002). Moreover, an SPN mutant that is incapable of binding to Impβ does not interfere with U1 import via the Sm core–dependent pathway (Ospina *et al.*, 2005). These observations show that the two import pathways are redundant in vitro, but they fail to elucidate the need for two independent snRNP import pathways. An in vivo model system of snRNP import is therefore needed to fully dissect the complex nature of this pathway.

As a first step toward developing an in vivo model system of snRNP nuclear import, we identified and characterized *Drosophila* Snurportin (dSNUP). We found that, like its human counterpart, dSNUP binds to snRNAs and to dSmB and dSMN in an RNA-dependent manner. Surprisingly, dSNUP lacks an obvious IBB domain and fails to bind to Impβ in vivo and in vitro. Furthermore, fruit fly Impβ does not interact with snRNAs. We also identified Moleskin (Msk), the *Drosophila* orthologue of the vertebrate transport factor importin-7 (Imp7), as the putative snRNP import receptor. Msk localizes to Cajal bodies and physically interacts with snRNPs. In addition, we discovered that Msk-null mutant larvae display a significant accumulation of TMG-capped RNAs in the cytoplasm of larval Malpighian tubules and reduced levels of snRNP biogenesis markers coilin and dSMN. These results demonstrate a novel and conserved interaction between Snurportin and Msk/Imp7. We discuss implications for studies of vertebrate nuclear import.

RESULTS

Identification and characterization of *Drosophila* Snurportin

Bioinformatic analysis of the *Drosophila* genome predicts that the fruit fly orthologue of human *Snurportin1* maps to the computed gene locus CG42303/CG42304, near band position 62E on chromosome 3L. The current FlyBase gene model predicts the existence of a dicistronic transcript with two nonoverlapping open reading frames (ORFs) present within CG42303. Reverse transcription (RT)-PCR and 5′-RACE data from the modEncode project (Sue Celniker lab; www.modencode.org/celniker/) support the existence of two transcription start sites (Figure 1A), one for each ORF. The protein predicted by CG42303 is encoded by a single exon and is 35% identical to SPN1. The CG42304 protein product is also highly similar (37% identity) to the human terminal deoxynucleotidyltransferase interacting factor 1 (TdIF1 or DNTTIP1), which binds and negatively regulates the activity of terminal deoxynucleotidyltransferase (Kubota *et al.*, 2007). This same genetic architecture exists in all other sequenced Drosophilid genomes but is not conserved in *Anopheles gambiae*, *Apis mellifera*, or *Caenorhabditis elegans*, as SPN1 and DNTTIP1 homologues are unlinked in these organisms. These data suggest that the two genes became linked sometime after the *Drosophila* radiation from other Diptera such as *Anopheles* or *Apis*.

Alignment of CG42303 with human SPN1 reveals extensive similarity throughout the length of the two sequences, especially within the TMG cap–binding domain (Huber *et al.*, 1998; Ospina *et al.*, 2005; Strasser *et al.*, 2005; and data not shown). Perhaps the most striking feature is that critical residues known to interact with Impβ in the N-terminal region of human SPN1 are missing from the putative fruit fly protein (Figure 1B; Ospina *et al.*, 2005). Human *SPN1* encodes a 360–amino acid (aa) protein of 43-kDa molecular weight; the SPN1-like ORF in CG42303 is predicted to generate a protein of 351 aa and 42 kDa. Using bacterially expressed protein targeting the upstream ORF in CG42303, we generated two polyclonal antibodies (one in rabbit, one in guinea pig) and tested them by Western blotting. As shown in Figure 2A (lane 1), the rabbit antiserum recognizes a prominent 42-kDa band, along with three other minor polypeptides. The guinea pig antiserum does not recognize endogenous snurportin in Western blots but does detect recombinant and exogenously expressed fly snurportin; it also works in other assays (see later discussion).

Previous gene models suggested different architectural scenarios for CG42303 and CG42304. One model posited the existence of two completely separate transcripts (CG1247, CG1248), whereas another (CG32297) suggested that there is a single mRNA that generates a fused ORF encoding a predicted protein of ~100 kDa. To examine the specificity of our antibody and test the various gene models, we designed double-stranded RNAs (dsRNAs) targeted against putative exons 1 and 3 of CG42303 (Figure 1A). RNA

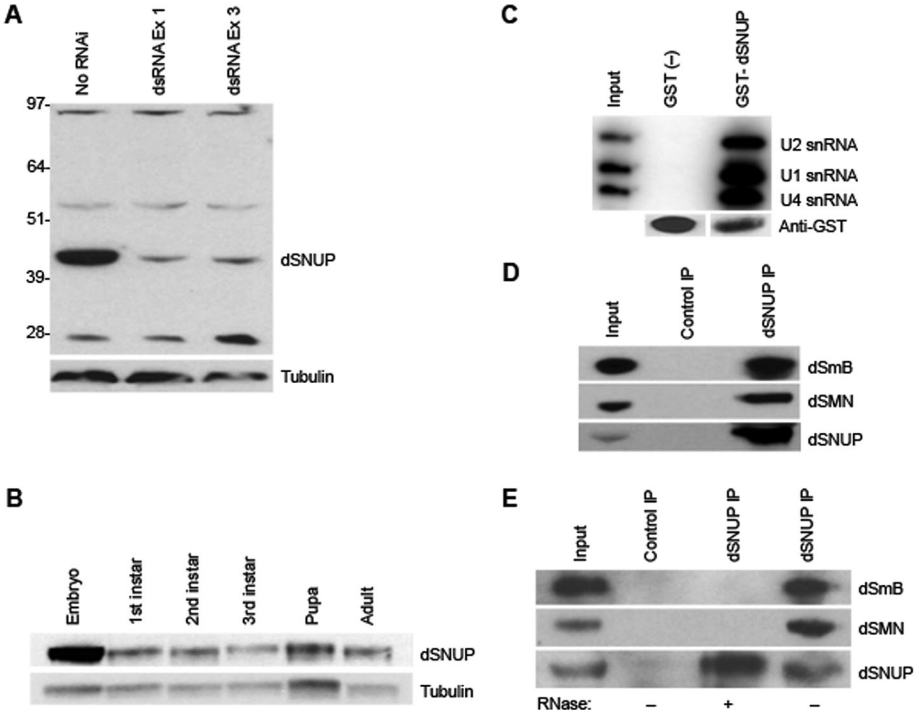


FIGURE 2: *Drosophila* Snurportin interacts with snRNPs. (A) dSNUP RNAi. Predicted 42-kDa band recognized by dSNUP rabbit antibody is specifically knocked down by dsRNAs targeting dSNUP exon 1 or 3 in S2 cell culture. (B) Developmental Western blot. dSNUP is expressed at all *Drosophila* developmental stages. (C) GST IP Northern blot. Bacterially purified GST-dSNUP interacts with U1, U2, and U4 snRNAs from S2 cell cytoplasmic lysate. (D) dSNUP Guinea pig IP. Guinea pig dSNUP antibody coimmunoprecipitates dSMN and dSmB in S2 cell cytoplasmic lysate. (E) RNase dSNUP Guinea pig IP. RNase treatment of cytoplasmic S2 lysate abolishes interaction of dSNUP with dSmB and dSMN.

interference (RNAi) analysis in *Drosophila* Schneider 2 (S2) cells shows that the 42-kDa band is specifically depleted by dsRNAs targeting either exon 1 or exon 3 (Figure 2A). This result supports the prediction that the CG42303 transcript contains a relatively long 3′ flanking region and shows that the 90-kDa band on the Western blot is not a fused SPN1/DNTTIP1-like chimeric protein. We conclude that the exonic organization in the CG42303 gene model is correct. The mRNA encoding the downstream CG42304 protein product is thus likely to originate from an alternative transcription start site (Figure 1A).

The CG42303 protein product is expressed during all stages of development, most prominently in embryos (Figure 2B). We found that although it does not work well for detection of endogenous dSNUP by Western blotting, guinea pig anti-dSNUP was functional in immunoprecipitation assays, as shown in Figure 2D. Using glutathione *S*-transferase (GST) pull downs and coimmunoprecipitation assays from S2 cell cytoplasmic lysates, we show that CG42303 interacts with both RNA and protein components of snRNPs, as well as with the snRNP biogenesis factor, dSMN (Figure 2, C and D). Furthermore, RNase treatment of the S2 lysate abolishes these protein interactions, demonstrating that they are RNA dependent (Figure 2E). These results provide strong evidence that CG42303 is the *Drosophila* orthologue of human SPN1. To avoid confusion with the abbreviations for the *Spinophilin* gene (*Spn*) and the *spindle* gene family (*spn-A*, *spn-B*, etc.) in *Drosophila*, we decided to designate the CG42303 gene as *Snurportin* (*Snup*).

Previously, we showed that human SPN1 primarily localizes to the cytoplasm, concentrating around the nuclear periphery and sometimes in nuclear Cajal bodies (Narayanan *et al.*, 2002; Ospina *et al.*, 2005). Using the UAS-Gal4 system (Brand and Perrimon, 1993), we expressed Venus fluorescent protein (VFP)–tagged dSNUP in transgenic flies and analyzed its localization by fluorescence microscopy. Using a variety of Gal4 drivers, we find that VFP-dSNUP localizes to the nucleus and the cytoplasm, with a pronounced accumulation at the nuclear periphery (Figure 3A). Of note, VFP-dSNUP localizes to snRNP-rich structures that costain with anti-dSmB in the oocyte germinal vesicle (Figure 3B) and in the nurse cell cytoplasm, where it accumulates in U bodies (Liu and Gall, 2007) identified by anti-dSMN (Figure 3C). In addition, dSNUP enrichment in U bodies was also confirmed in the follicle cell cytoplasm of egg chambers, visualized by anti-dSmB (Figure 3D). In larval Malpighian tubules, VFP-dSNUP frequently localizes to Cajal bodies (Figure 3A). This localization pattern is similar to that of human SPN mutants that contain deletions or substitutions in the IBB domain (Narayanan *et al.*, 2002; Ospina *et al.*, 2005). We therefore decided to examine the interaction between dSNUP and Ketel/Impβ.

Drosophila snRNP import is importin-β independent

Studies in vertebrates show that SPN1 interacts with Impβ and that this interaction is mediated via the IBB domain (Huber *et al.*, 1998, 2002; Bhardwaj and Cingolani, 2010). The bipartite IBB of SPN1 is contained within residues 1–65 (Mitrousis *et al.*, 2008), and crystal structures reveal that residues 1–16 also contain a nuclear export signal recognized by the export receptor, Xpo1/Crm1 (Dong *et al.*, 2009; Monecke *et al.*, 2009). Sequence analysis indicates that dSNUP lacks important residues in the IBB (Figure 1B; Huber *et al.*, 2002; Ospina *et al.*, 2005; Mitrousis *et al.*, 2008), suggesting that it might not bind to Impβ. Specifically, a highly conserved arginine residue, mutation of which disrupts the interaction of SPN1 with Impβ (Ospina *et al.*, 2005), is not conserved in dSNUP (Figure 1B, asterisk). In the absence of an IBB, dSNUP could potentially interact with Ketel/Impβ indirectly through the Sm core (Fischer *et al.*, 1993). Human SPN1 also forms a preimport snRNP complex with SMN (Narayanan *et al.*, 2002). To enrich for import competent assemblies, we used cytoplasmic extracts to carry out immunoprecipitation and pull-down assays. As a positive control for coimmunoprecipitation, we show that, like its human counterpart, dSNUP forms a complex with dSMN (Figure 4A). Consistent with its lack of an apparent IBB domain, however, dSNUP fails to coimmunoprecipitate Ketel/Impβ (Figure 4A).

We also found that Ketel is capable of interacting with an IBB domain by transfecting S2 cells with various Flag-tagged constructs and coexpressing them with green fluorescent protein (GFP)–Ketel. As shown in Figure 4B, Flag-tagged human SPN1 (Flag-hSPN) or the human SPN1 IBB domain fused to the TMG cap–binding domain of dSNUP (Flag-hIBB-dSNUP) coimmunoprecipitates GFP-Ketel, whereas the empty Flag vector (negative control) and Flag-dSNUP do not. Finally, we tested whether Ketel interacts with snRNAs.

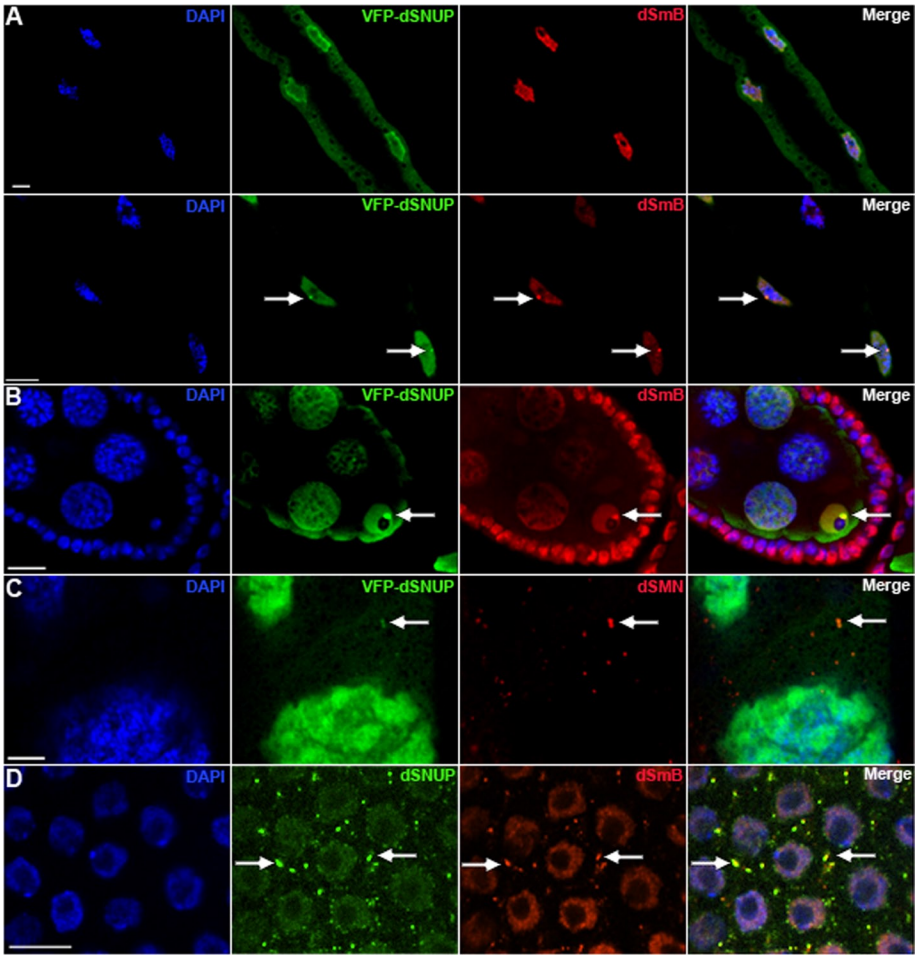


FIGURE 3: Localization of dSNUP. (A) Immunofluorescence with dSmB (Y12) antibody in Malpighian tubules expressing VFP-dSNUP driven by tubulin-Gal4. dSNUP localizes primarily to the nucleus, with a relatively pronounced staining of the nuclear periphery, and can be found in nuclear foci that are often Cajal bodies (marked by arrows). (B) Immunofluorescence with dSmB (Y12) in egg chambers expressing VFP-dSNUP driven by nanos-Gal4. VFP-dSNUP is enriched in the germinal vesicle (arrow). (C) Immunofluorescence with dSMN antibody in egg chambers expressing VFP-dSNUP driven by nanos Gal4. VFP-dSNUP is enriched in U bodies visualized with dSMN antibody (arrow). (D) Immunofluorescence with dSmB (Y12) and dSNUP Guinea pig antibodies in egg chambers. dSNUP is enriched in U bodies of follicle cells. Image in D was kindly provided by Zhipeng Lu (University of North Carolina, Chapel Hill). Scale bars, 10 μm.

Immunoprecipitation analysis, followed by Northern blotting, showed that, whereas GFP-dSNUP coimmunoprecipitated U1, U2, and U4 snRNAs (Figure 4C), GFP-Ketel failed to do so. Thus neither the RNA nor the protein components of snRNPs interact with Ketel in *Drosophila*. Taken together with the fact that we were unable to detect Ketel in a complex with dSNUP, these experiments strongly support the interpretation that Ketel does not serve as the snRNP import receptor in *Drosophila* cells.

Moleskin/importin-7 interacts with snRNPs and Snurportin

The failure of Ketel/Impβ to associate with dSNUP or snRNAs suggests the involvement of another import factor. Because splicing is a cell essential function, we reasoned that potential snRNP import receptors not only must be ubiquitously expressed, but also should be able to function independently of Impβ. Of interest, Paraskeva *et al.* (1999) originally showed that epitope-tagged human SPN1 copurifies with three major proteins: Impβ, the export receptor CRM1, and the transport factor Imp7. The authors went on to show that CRM1 functions as the cytoplasmic recycling factor for SPN1 once it deposits its cargo in the nucleus (Paraskeva *et al.*, 1999). The interaction between SPN1 and Imp7, however, was believed to be indirect due to the fact that Imp7 (formerly RanBP7) was shown to heterodimerize with Impβ (Görlich *et al.*, 1997). Imp7 also binds directly to the nuclear pore complex (Görlich *et al.*, 1997), however, and can transport cargoes independently (Jäkel and Görlich, 1998), thus satisfying an important criterion noted earlier.

The *Drosophila* Imp7 homologue (Moleskin [Msk]) is 53% identical to the human protein and was identified in a dominant suppressor screen for wing blisters caused by the misexpression of αPS integrin (Baker *et al.*, 2002). All of the alleles that were discovered in this suppression screen (*msk*^{2}, *msk*^{4}, *msk*^{5}) are late embryonic or larval lethal. It is interesting to note that although *msk* and *ketel* are both essential genes, there are cell types in which Ketel expression is very low (FlyBase; Lippai *et al.*, 2000). On the other hand, Msk is ubiquitously expressed (FlyBase), satisfying the other aforementioned criterion. Thus it is possible that Msk/Imp7 plays a more direct role in snRNP import than previously imagined.

To investigate whether Imp7/Msk forms complexes with snRNP biogenesis markers, we carried out coimmunoprecipitation analyses. As shown in Figure 5A, anti-dSNUP coprecipitates Msk; dSMN and Ketel are shown as positive and negative controls, respectively. S2 cells transfected with various GFP-tagged constructs also coprecipitated Msk. Figure 5B shows that Msk interacts with GFP-dSNUP, -dSMN, and -dSmB. GFP-Msk also coprecipitated with U1, U2, and U4 snRNAs, as shown in Figure 5C. In addition, RanQ69L (a Ran mutant that is unable to hydrolyze bound GTP; Bischoff *et al.*, 1994) disrupts the interaction of Flag-dSNUP with Msk (Figure 5D). This interaction also depends on RNA, as RNase treatment of cytoplasmic lysate abolishes binding of endogenous Msk to either GFP-dSNUP or GFP-dSMN (Figure 5E). These results clearly demonstrate that Msk can physically interact with snRNPs and that Msk interacts with dSNUP in a Ran- and RNA-dependent manner.

Moleskin/importin-7 localizes to snRNP-rich structures in the nucleus

As a nucleocytoplasmic transport factor, Msk shuttles from the cytoplasm to the nucleus. As such, previous investigations showed that the subcellular localization of Msk (also known as DIM-7) is dynamic; in certain cells the protein was primarily found in the cytoplasm, whereas in others it was predominantly nuclear (Lorenzen *et al.*, 2001; James *et al.*, 2007). Given that Msk forms complexes with snRNP components and biogenesis factors, we carried out immunofluorescence analyses in *Drosophila* larval and adult tissues. Msk is primarily cytoplasmic within the egg chambers of the

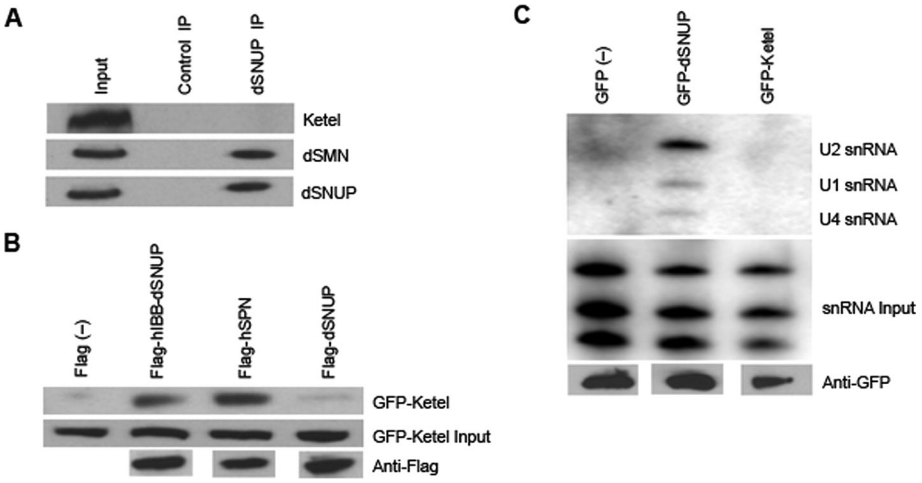
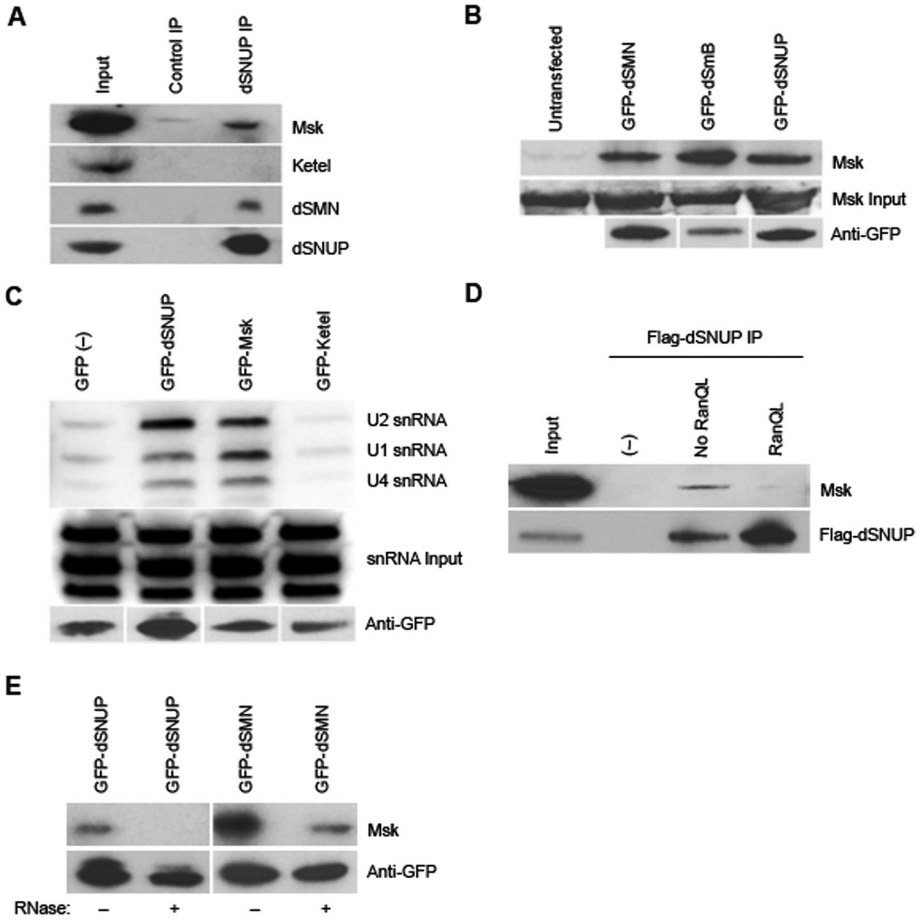


FIGURE 4: Ketel/Impβ does not interact with *Drosophila* snRNPs. (A) Anti-dSNUP guinea pig IP Western blot. dSNUP guinea pig antibody does not coimmunoprecipitate Ketel from cytoplasmic S2 cell lysate. (B) Flag-conjugated beads IP Western blot. Transfected Flag-tagged proteins hSPN and hIBB-dSNUP, but not dSNUP, coimmunoprecipitate GFP-Ketel in S2 cell cytoplasmic lysate. The amounts of Flag-tagged proteins immunoprecipitated are shown with anti-Flag (bottom). (C) GFP IP Northern blot. Transfected GFP-dSNUP coimmunoprecipitates snRNAs U2, U1, and U4, but GFP-Ketel does not, from S2 cell cytoplasmic lysate. The amounts of GFP-tagged proteins immunoprecipitated are shown with anti-GFP (bottom).

In mammalian cells, Cajal bodies are the first detectable sites of nuclear accumulation of newly imported snRNPs (Sleeman and Lamond, 1999). Given the interaction data described earlier, we suspected that these Msk-positive nuclear foci were Cajal bodies. Indeed, costaining for Sm proteins and other Cajal body markers demonstrates that the foci correspond to Cajal bodies (Figure 6 and data not shown). Note that the oocyte nucleoplasm typically does not stain uniformly with 4′,6-diamidino-2-phenylindole (DAPI); only the karyosome is well stained (Liu *et al.*, 2006a). Within the germinal vesicle, Cajal bodies can often be found proximal to the karyosome (Figure 6B), although they can also be distally located (Figure 6A). Owing to their relative prominence within larval Malpighian tubule nuclei, Cajal bodies are perhaps best visualized in this tissue (Liu *et al.*, 2006b). In Malpighian tubules (Figure 6C), we found that Msk is primarily nucleoplasmic and accumulates in bright nuclear foci. The bright Msk foci colocalize with the snRNP core component, dSmB (Figure 6C) or coilin (data not shown). In S2 cell cultures, only a fraction of the cells display Cajal bodies. Whenever we observed the bright nuclear foci that stained with anti-Msk, however, they invariably also stained positive for coilin, the Cajal body marker protein (Figure 6D). These results provide strong support for the notion that Msk is involved in import of Sm-class snRNPs.

ovary (Figure 6A), but both nurse and follicle cell nuclei remain largely unstained. Msk also shows prominent localization to the nurse cell nuclear periphery and to bright foci within the oocyte germinal vesicle (Figure 6A).



Moleskin depletion disrupts snRNP biogenesis and import

RNA interference (RNAi) analysis in S2 cells using dsRNAs targeting Msk revealed that Cajal bodies were disrupted by Msk depletion (data not shown). This finding is consistent with previous results showing that Cajal body homeostasis requires ongoing

FIGURE 5: Moleskin interacts with *Drosophila* snRNPs. (A) dSNUP Guinea pig IP Western blot. dSNUP Guinea pig antibody coimmunoprecipitates Msk but not Ketel from S2 cell cytoplasmic lysate. (B) GFP IP Western blot. Msk coimmunoprecipitates with transfected GFP-dSMN, GFP-dSmB, and GFP-dSNUP from S2 cell cytoplasmic lysate. (C) Anti-GFP IP Northern blot. Major U snRNAs U1, U1, and U4 coimmunoprecipitate with GFP-Msk and GFP-dSNUP but not GFP-Ketel. (D) Anti-Flag IP Western blot. Transfected Flag-dSNUP coimmunoprecipitates Msk in the absence of RanQL. This interaction is disrupted by the addition of RanQL. Nonconjugated protein A beads serves as negative control IP (–). (E) RNase anti-GFP IP Western blot. RNase treatment of cytoplasmic S2 lysate abolishes interaction of transfected GFP-dSNUP and GFP-dSMN with endogenous Msk.

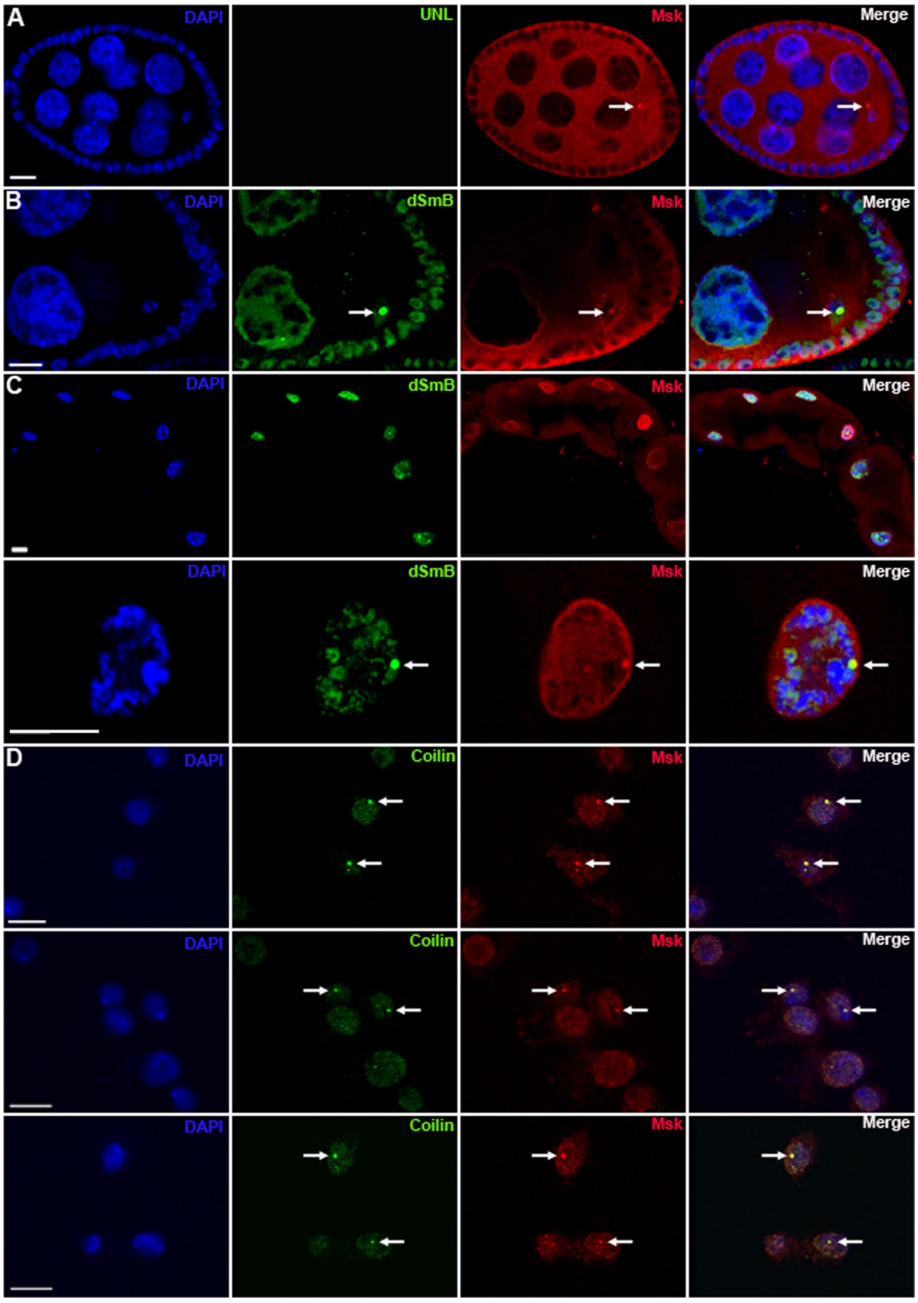


FIGURE 6: Moleskin is enriched in Cajal bodies. (A) Immunofluorescence in egg chambers with Msk antibody. Msk is enriched in the germinal vesicle (arrow). (B) Immunofluorescence in egg chambers with dSmB (Y12) and Msk antibodies. Msk is enriched in the germinal vesicle (arrow). (C) Immunofluorescence in Malpighian tubules with dSmB (Y12) and Msk antibodies. Msk is enriched in Cajal bodies of Malpighian tubules. (D) Immunofluorescence in S2 cells with coilin and Msk antibodies. Msk is enriched in Cajal bodies of S2 cells. Scale bars, 10 μm.

snRNP biogenesis (Shpargel and Matera, 2005; Lemm *et al.*, 2006). Because U snRNPs are extremely stable complexes, however, with half-lives on the order of 3–5 d (Sauterer *et al.*, 1988), this analysis was not very informative with regard to snRNP phenotypes. We therefore obtained a presumptive *msk*-null mutant from the Bloomington *Drosophila* Stock Center ($msk^{-/-}$), which contains a *piggyback* transposon insertion in exon 1. We confirmed by Western blotting that this allele is indeed a null, demonstrating the absence of Msk protein in homozygous mutant larvae (Figure 7A).

Moleskin-null mutants are larval lethals (Lorenzen *et al.*, 2001); a small fraction of mutant larvae survive >10 d, but they do not develop past the second instar. The extended survival of *msk* mutants suggests that, like Ketel protein (Villányi *et al.*, 2008), Msk protein also has a long half-life.

To determine whether there are snRNP-specific phenotypes associated with loss of Msk, we carried out immunofluorescence with anti-TMG cap antibodies. Wild-type, $Ketel^{null/-}$, and UAS-*msk* transgenic rescue animals were used as controls. Moleskin mutants displayed a slight but reproducible cytoplasmic TMG accumulation in the Malpighian tubules (Figure 8), suggesting a disruption in snRNP import and/or biogenesis. This accumulation was not simply due to the developmental arrest, as $Ketel^{null/-}$ mutants do not display this phenotype, and expression of UAS-*msk* rescues it (Figure 8). In an effort to bypass the Msk dependence of this observed snRNP import defect, we generated transgenic flies expressing VFP-dSNUP or VFP-hIBB-dSNUP from UAS promoters. Because we previously showed that hIBB-dSNUP forms a complex with Ketel (Figure 4B), we hypothesized that its expression might rescue snRNP import in Malpighian tubules. We therefore expressed these transgenes in both wild-type and $msk^{-/-}$ backgrounds. Using either a ubiquitous tubulin-Gal4 driver or a gut-specific Malpighian tubule driver, we found that expression of VFP-hIBB-dSNUP was dominantly lethal in both backgrounds. It is unlikely that the dominant-negative phenotype of the hIBB-dSNUP construct is due to VFP tagging because expression of the control VFP-dSNUP construct had no such dominant effects and was able to rescue dSNUP RNAi (data not shown). The dominant lethality of the hIBB-dSNUP fusion precluded us from testing whether targeting dSNUP to an alternative nuclear import receptor pathway (in this case Ketel) might alleviate the apparent block to snRNP import.

We therefore conducted immunofluorescence with anti-dSMN and anti-coilin antibodies in control and *msk* mutant larvae. Confirming the results noted for S2 cells, we found that in the Malpighian tubules of *msk* mutants, dSMN and coilin staining was dramatically reduced and Cajal bodies were disrupted (Figure 9). Staining for both dSMN and Cajal bodies (anti-coilin) was restored upon expression of (untagged) Msk using a UAS-*msk* transgene (Figure 9). As shown in Figure 7B, the loss of dSMN is fairly extensive, as it can be detected by Western blotting using total larval lysates. Of importance, the expression of UAS-*msk* transgene partially rescues both Msk and dSMN expression (Figure 7, B and C), as well as development of the organism beyond larval stages (Lorenzen *et al.*, 2001; this work). In addition, coilin and dSMN reduction is detectable by day 1 post egg laying

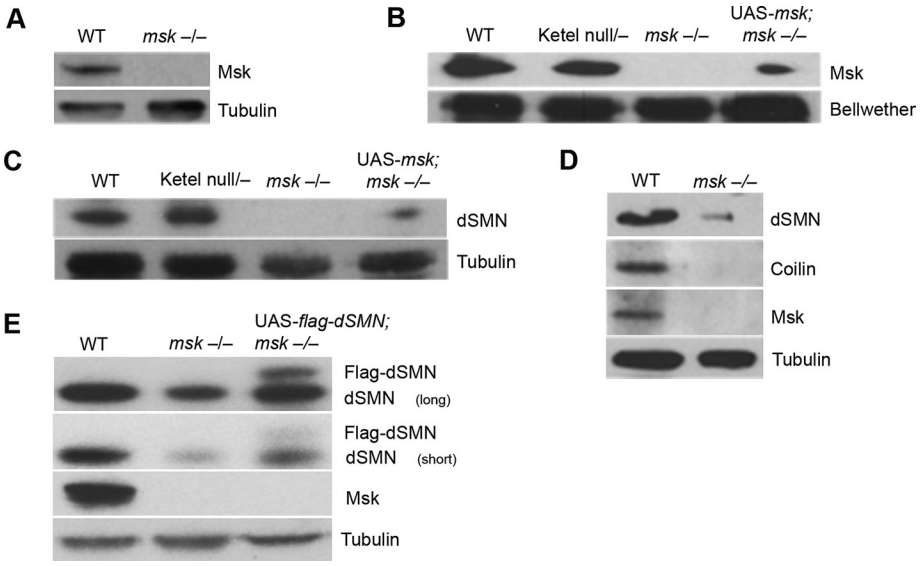


FIGURE 7: Moleskin mutant characterization. (A–C) Western blot of second-instar larvae. (A) *msk*$^{-/-}$ larvae have significantly reduced Msk protein levels. (B) Ketel$^{null/-}$ larvae have WT levels of Msk. UAS-*msk* driven by armadillo-Gal4 in the *msk*$^{-/-}$ background shows recovery of Msk protein. (C) *msk*$^{-/-}$ larvae have significantly reduced levels of dSMN, which can be recovered by UAS-*msk* driven by armadillo-Gal4. (D) Western blot of first-instar larvae. *msk*$^{-/-}$ larvae have significantly reduced levels of dSMN and coilin by day 1 post egg laying (DPE). (E) Western blot of first-instar larvae. UAS-*flag-dSMN* driven by armadillo-Gal4 in the *msk*$^{-/-}$ background shows Flag-dSMN expression. Long exposure (top) and short exposure (middle) with dSMN antibody.

(Figure 7D). Thus Msk is required for the stability of dSMN and coilin.

SMN plays a crucial role in snRNP biogenesis, and its depletion disrupts Cajal bodies in HeLa cells (Shpargel and Matera, 2005). Therefore the significant reduction of dSMN in Msk mutant larvae could be responsible for the Cajal body and TMG cap phenotypes. To investigate this possibility, we overexpressed Flag-tagged dSMN in the Msk-mutant background (Figure 7E). Overexpression of Flag-dSMN failed to rescue organismal viability, Cajal body disruption, or cytoplasmic TMG cap localization (Figure 10). Therefore Msk function in vivo is not limited to SMN stability. Taken together with the subcellular localization and biochemical interaction analyses described earlier, these genetic results provide strong evidence linking Msk to a role in snRNP biogenesis.

DISCUSSION

Vertebrate Imp7 and Impβ form an abundant heterodimeric complex (Görlich *et al.*, 1997). Because Impβ is entirely sufficient for snRNP import in vitro and in *Xenopus* oocytes (Huber *et al.*, 1998; Palacios *et al.*, 1997), it was assumed that the copurification of Imp7 with SPN1 in HeLa cells was simply an indirect consequence of its interaction with Impβ (Paraskeva *et al.*, 1999). In this study, we show that dSNUP is the *Drosophila* orthologue of human SPN1 and provide convincing evidence that it fails to bind Ketel/Impβ in vitro and in vivo. Our results strongly favor the interpretation that *Drosophila* snRNP import uses the import receptor Msk/Imp7 in place of Ketel/Impβ. Thus the physical interaction between Imp7/Msk and SPN1/dSNUP is conserved in humans and *Drosophila*, raising the question of whether Imp7 might play a previously unrecognized role in vertebrate snRNP import.

In mammalian cells, Imp7 functions as an import receptor for various protein cargoes, independent of its role as an adaptor for Impβ (Jäkel *et al.*, 1999; Freedman and Yamamoto, 2004). Thus it is possible that Imp7 plays a similar role in the snRNP import pathway in mammals. Previous results from our lab show that SMN can bind directly to Impβ in vitro and that purified SMN complexes are required for SPN1 independent snRNP import (Narayanan *et al.*, 2004). The precise identity of the import adaptor for the Sm-core mediated import pathway, however, is not known. Whether the Impβ-binding site of SMN is masked while the protein is in the SMN import complex is also unknown. Several possibilities thus exist in vivo: Impβ may bind directly to SMN or indirectly through an unidentified adaptor protein (e.g., Imp7/Msk), or some combination of both scenarios might hold, as they are not mutually exclusive.

We envision two models by which Imp7 could function in the nuclear import of snRNPs in vertebrates. In one scenario, Imp7 and Impβ could have partially redundant functions, in which they could each independently function as import receptors in single snRNP import events (Figure 11A). Alternatively, Imp7 could serve as an import adaptor for Impβ, functioning with it in the same import cycle (Figure 11B). Curiously, we find that an unidentified band of the appropriate size copurifies with the SMN complex in numerous publications (Baccon *et al.*, 2002; Pellizzoni *et al.*, 2002; Yong *et al.*, 2004; Carissimi *et al.*, 2005, 2006a,b). Thus it is possible that Imp7 is the unidentified Sm core import adaptor

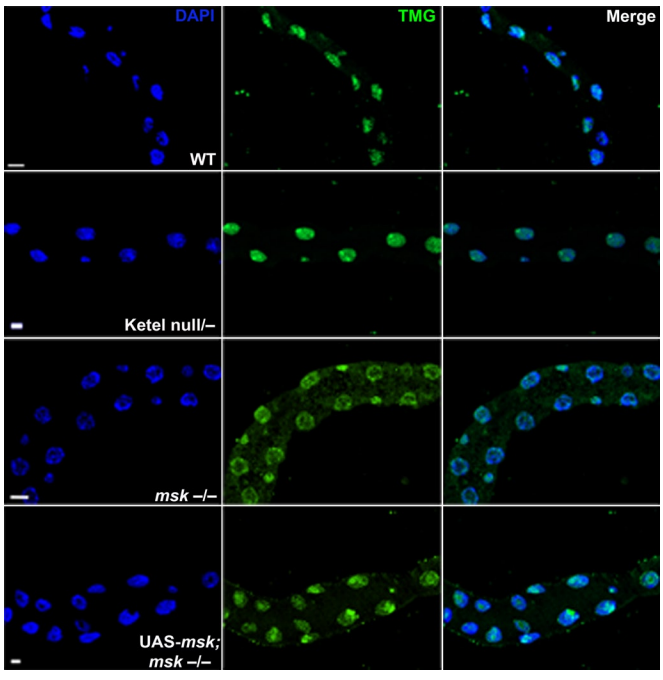


FIGURE 8: Moleskin-null mutant Malpighian tubules display TMG cap cytoplasmic accumulation. Immunofluorescence in second-instar larvae. Long-lived *msk*$^{-/-}$ larvae show cytoplasmic accumulation of TMG in Malpighian tubules, whereas similar long-lived Ketel$^{null/-}$ larvae do not. UAS-*msk* driven by armadillo-Gal4 in the *msk*$^{-/-}$ background partially rescues cytoplasmic TMG phenotype. Scale bars, 10 μm.

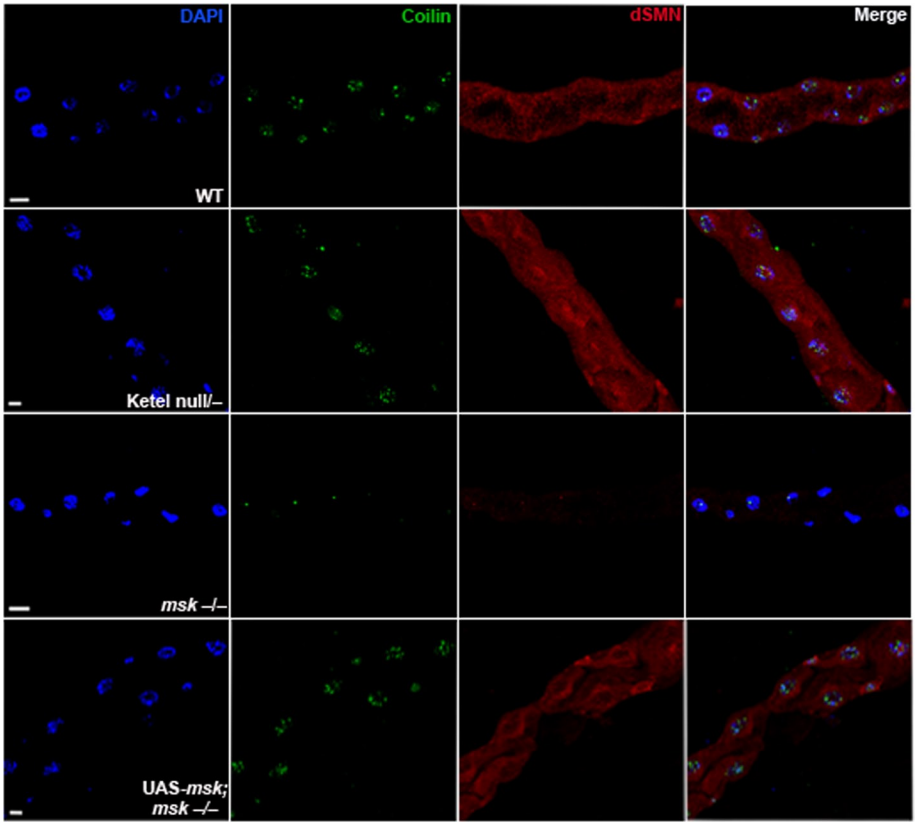
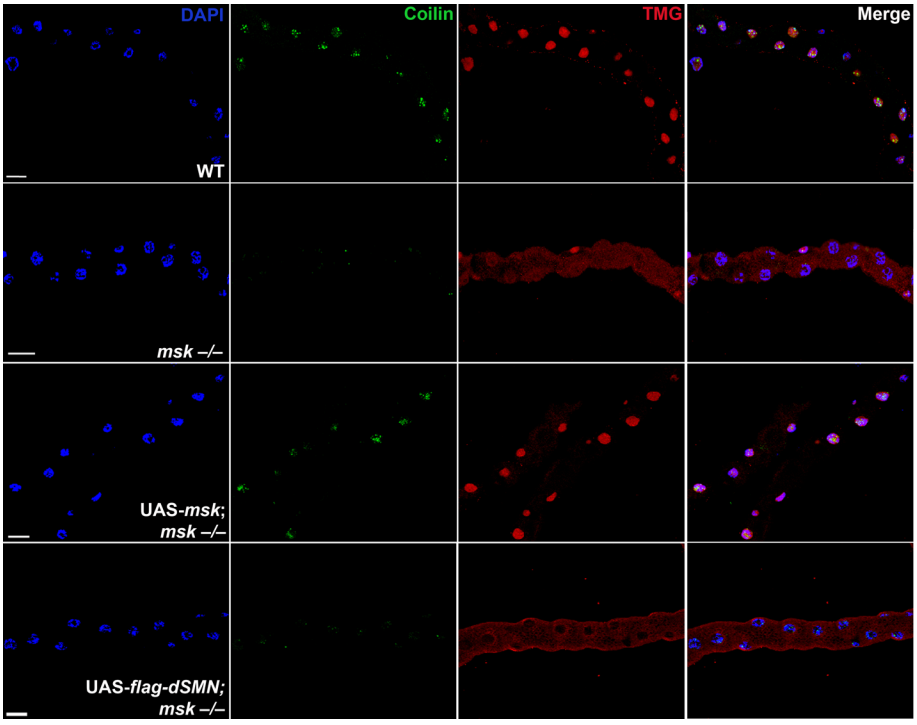


FIGURE 9: Coilin and dSMN are reduced in Moleskin-mutant Malpighian tubules. Immunofluorescence in second-instar larvae. Long lived $msk^{-/-}$ larvae have reduced dSMN and Cajal bodies (anti-coilin) in Malpighian tubules, whereas similar long-lived $Ketel^{null/-}$ larvae do not. UAS-*msk* driven by armadillo-Gal4 in the $msk^{-/-}$ background shows recovery of both coilin and dSMN (UAS-*msk*; $msk^{-/-}$). Scale bars, 10 μm.



protein in vertebrates, definitive identification of which remains a subject of considerable interest.

Traditionally, import receptors have been believed to be bound immediately by RanGTP in the nucleus; subsequently the receptors are recycled back into the cytoplasm. There is evidence, however, that Imp7 may be a bit different from traditional nuclear import receptors. Unlike Impβ, Imp7 does not require RanGTP for histone H1 nuclear import (Jäkel *et al.*, 1999). The lower affinity for RanGTP is hypothesized to be a potential advantage. Jäkel *et al.* (1999) suggested that by delaying the dissociation of Imp7 from H1, Imp7 could accompany the histone to the chromosome for assembly into chromatin. The same idea could be applied to our surprising finding that Msk/Imp7 localizes to Cajal bodies in both *Drosophila* and human cells (Figure 6 and Supplemental Figure S1, respectively). Hence Msk/Imp7 might act in a chaperonin-like manner inside the nucleus, ferrying snRNPs to Cajal bodies for potential interaction with coilin and/or SMN (Liu *et al.*, 2000; Narayanan *et al.*, 2004; Ospina *et al.*, 2005; Shpargel and Martel, 2005; Tanackovic and Kramer, 2005).

Navigating the complex nature of snRNP import mechanisms will require precise molecular dissection of the interactions between snRNPs, their transport receptors, and their downstream effectors. Our finding that loss of *msk* function leads (directly or indirectly) to codepletion of dSMN is particularly significant in this regard. Collectively, our studies provide strong evidence that Ketel/Impβ is not the TMG cap import receptor in *Drosophila* and that Msk/Imp7 is required for ongoing snRNP biogenesis. Furthermore, we provide important food for thought regarding a potential role for Imp7/Msk in mammalian snRNP import. Imp7/Msk may have different binding capacities than Impβ/Ketel in particular tissues or for individual species of U snRNPs. Additional experiments are needed to clarify these and other important questions. Understanding the role of

FIGURE 10: Overexpression of Flag-dSMN does not rescue coilin and dSMN reduction in Moleskin-mutant Malpighian tubules. Immunofluorescence in second-instar larvae. Long-lived $msk^{-/-}$ larvae have reduced dSMN and Cajal bodies (anti-coilin) in Malpighian tubules compared with wild type. UAS-*msk* driven by armadillo-Gal4 in the $msk^{-/-}$ background shows recovery of both coilin and dSMN (UAS-*msk*; $msk^{-/-}$). UAS-*flag-dSMN* driven by armadillo-Gal4 in the $msk^{-/-}$ background fails to rescue coilin or dSMN (UAS-*flag-dSMN*; $msk^{-/-}$). Scale bars, 20 μm.

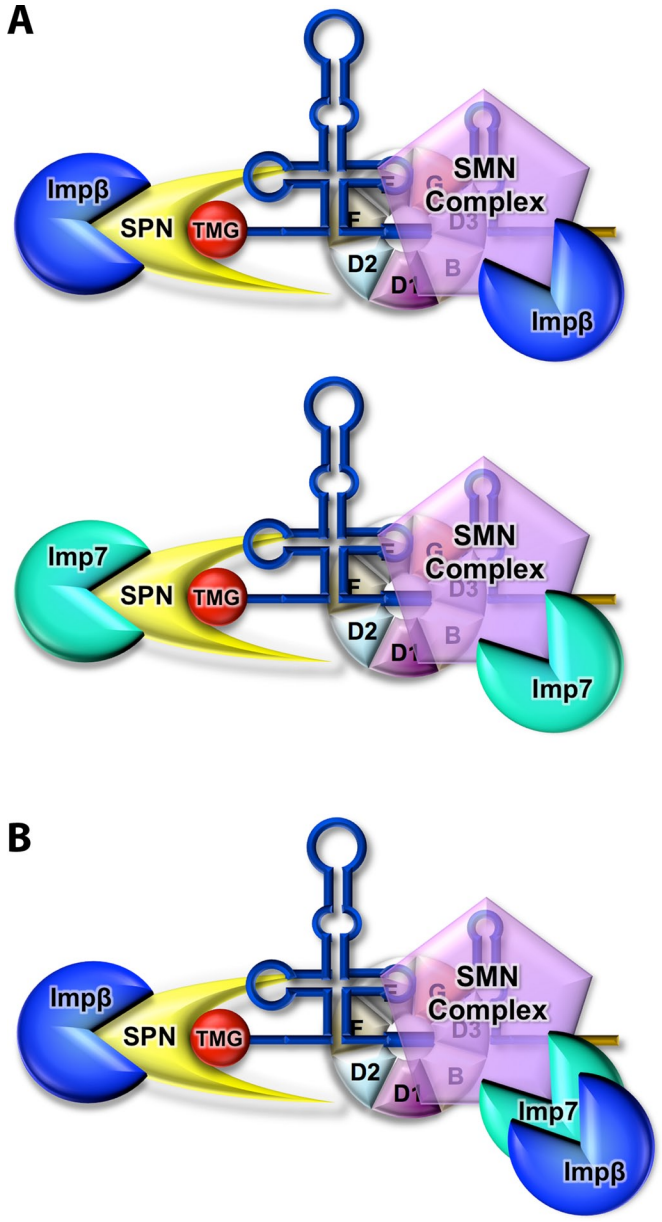


FIGURE 11: Models of Imp7 role in snRNP import. (A) Imp7 and Impβ could function redundantly as autonomous snRNP import receptors. (B) Alternatively, Imp7 could function as an Sm core snRNP import adaptor for Impβ.

Imp7/Msk in snRNP biogenesis in both vertebrate and invertebrate systems should elucidate how the two different nuclear import pathways complement one another.

MATERIALS AND METHODS

DNA constructs

dSNUP, *hIBB-dSNUP*, *dSMN*, *dSmB*, *Msk*, and *Ketel* full-length cDNAs were PCR amplified with appropriate primers flanked by Gateway recombination sequences (Invitrogen, Carlsbad, CA). These products were recombined initially into pDONR221 (Invitrogen) before entry into GFP-tagged pAGW, Flag-tagged pAFW (*Drosophila* Genome Research Center, Indiana University, Bloomington, IN), or *pBI-UASC-mVenus* (a gift from Brian McCabe, Columbia University, New York, NY).

Recombinant protein expression and S2 cell transfections

GST-dSNUP was expressed in BL21-star bacteria (Invitrogen) by 1 mM isopropyl-β-D-thiogalactoside induction for 3 h. Lysate was extracted by sonication and passed over glutathione beads. S2 cells were transfected using Cellfectin as directed (Invitrogen), and cells were harvested 4 d after transfection.

Antibodies

A rabbit polyclonal anti-dSNUP antibody (dSNUP) was generated (Pacific Immunology, Ramona, CA) using GST-tagged dSNUP. A guinea pig polyclonal anti-dSNUP antibody was generated (Pocono Rabbit Farm and Laboratory, Canadensis, PA) using dSNUP.

GST (Santa Cruz Biotechnology, Santa Cruz, CA; anti-mouse; 1:1000), GFP (Roche, Indianapolis, IN; anti-mouse; 1:1000, and Abcam, Cambridge, MA; anti-rabbit, 1:1000), dSmB (Y12) anti-sDMA (a gift from J. Steitz, Yale University, New Haven, CT; anti-mouse; 1:3000), dSMN (Praveen *et al.*, 2012; affinity-purified anti-rabbit; 1:2000), dSNUP (affinity-purified anti-rabbit; 1:3000), Msk (a gift of L. Perkins, Harvard Medical School Boston, MA; anti-rabbit; 1:2000), Ketel (a gift from J. Szabad, Faculty of Medicine, University of Szeged, Szeged, Hungary; anti-rabbit; 1:5000), bellwether (Abcam, Cambridge, MA; anti-mouse, 1:5000), Flag (Sigma-Aldrich, St. Louis, MO; horseradish peroxidase–conjugated anti-Flag; 1:8000), and tubulin (Sigma-Aldrich; anti-rabbit; 1:10,000) antibodies were used for Western blotting. Secondary antibodies used were goat anti-mouse–, anti–guinea pig–, and anti-rabbit–conjugated horseradish peroxidase at 1:5000 (Pierce, Rockford, IL).

Msk (a gift of L. Perkins; rabbit; 1:1000), coilin (a gift of J. Gall, Carnegie Institution for Science, Baltimore, MD; guinea pig; 1:1000), dSMN (Praveen *et al.*, 2012; affinity-purified rabbit; 1:200), dSNUP (guinea pig; 1:200), dSmB (Y12) anti-sDMA (a gift from J. Steitz, Yale University, New Haven, CT; mouse monoclonal; 1:200), Imp7 (Sigma-Aldrich; rabbit; 1:250), and hSMN (mouse monoclonal; clone 8, BD Biosciences, San Diego, CA; 1:250) were used for immunofluorescence. GFP (Abcam, Cambridge, MA; rabbit; 1.5 µl), GFP (mouse; Roche; 1.5 µl), and dSNUP (guinea pig; 10 µl) antibodies and Flag-conjugated agarose beads (Sigma-Aldrich; 15 µl per immunoprecipitation [IP]) were used for IP in buffer A (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol).

Immunoprecipitation

S2 cell cytoplasmic lysate was prepared by resuspending cells in 5× pellet volume of buffer A. Resuspended cells were incubated on ice for 30 min to allow swelling, mixed 10× with a p200 pipette, and incubated for an additional 10 min on ice before passing through a 27.5-gauge needle 40×. Cells were spun for 1 min at 13,000 rpm in a microfuge, and the cytoplasmic supernatant was treated with protease inhibitor cocktail (Pierce). For RNase experiments, S2 cell cytoplasmic lysate was divided into equal fractions, which were untreated or treated with 1 µg of RNase/5 µg lysate for 1 h at 37°C. For RanQ69L experiments, bacterially expressed GST-RanQ69L was added to cytoplasmic lysate. Cytoplasmic fractions were incubated with antibody for 1 h (no antibody added for negative control IP) at 4°C before incubation overnight at 4°C with 15 µl of protein A beads (Pierce). Bound proteins were washed 5× with 1 ml of buffer A.

For IP Northern experiments, bound RNA was directly phenol/chloroform extracted off beads, denatured in formamide loading buffer, run on a 10% polyacrylamide-urea gel (Invitrogen), transferred to a nylon membrane, and probed with ^{32}P-labeled PCR products corresponding to the *D. melanogaster* U1, U2, and U4 snRNAs.

RNAi

dSNUP dsRNAs were transcribed in vitro from PCR products flanked with T7 promoters. *Drosophila* S2 cells were placed in SF-900 media and treated with fresh 14 µg/ml dsRNA each day for 4 d before harvesting. Cytoplasmic extracts were generated 4 d after transfection. A 50-µg portion of cytoplasmic extract was loaded on a polyacrylamide gel for Western blotting analysis to confirm knockdown.

Fly stocks

Oregon-R was used as the wild-type strain. A Msk-null line containing a piggyback insertion in intron 1 of Msk (*msk*$^{-/-}$), Msk^{B185}, w^{1118}; PBac{5HPw^{+}}msk^{B185}/TM3, Sb^{1} Ser^{1}, and a line containing *msk* with a UAS promoter (UAS-*msk*), w*; P{UAS-*msk*.L}47M1/CyO, previously characterized (Lorenzen *et al.*, 2001), were obtained from the Bloomington *Drosophila* Stock Center (Bloomington, IN). Armadillo-Gal4 was recombined with *msk*$^{-/-}$ and crossed to UAS-*msk* for rescue of previously characterized UAS-*flag-dSMN* (Chang *et al.*, 2008). Previously characterized Ketel$^{null/-}$ (Villányi *et al.*, 2008) was a gift from J. Szabad. (The – sign stands for a small deficiency [ketel^{rx32}] that removes Ketel and a few of the adjacent loci, whereas the Ketel null [ketel^{rx13}] is a complete loss-of-function mutant allele; Erdélyi *et al.*, 1997.)

The *dSNUP* and *hIBB-dSNUP* transgenic constructs were cloned into *pBI-UASC-mVenus* (Wang *et al.*, 2012) and sent to BestGene (Chino Hills, CA) for embryo injection using the phiC31 system. Transgenes were integrated at site 86fB (Bischof *et al.*, 2007). Flies bearing a UAS:VFP-*Snup* transgene were crossed to a variety of Gal4 drivers, including tubulin-Gal4 and nanos-Gal4. The *msk*$^{-/-}$ flies were recombined with either *VFP-dSNUP* or *VFP-hIBB-dSNUP* transgenic lines and with Gal4 drivers. Timed matings were allowed to proceed for 6 h, and larvae were collected for phenotypic analyses on subsequent days.

Immunofluorescence

Drosophila tissues and HeLa and S2 cells were fixed at room temperature for 10 min in 3.7% paraformaldehyde in phosphate-buffered saline (PBS; 135 mM NaCl, 2.5 mM KCl, 4.3 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.2). Tissues/cells were then permeabilized with 1% Triton 100×, blocked in PBST (PBS with 0.1% Triton 100×) containing 5% NGS (blocking solution) and then washed with PBST. The primary antibody, diluted in PBST, was incubated with the samples overnight at 4°C. After being washed with PBST, the secondary antibody, diluted in blocking solution, was incubated with the samples for 2 h at room temperature. The samples were stained with DAPI, washed with PBST, and mounted in antifade solution (0.233 g of DABCO, 800 µl of water, 200 µl of 1 M Tris-HCl, pH 8.0, 9 ml of glycerol).

Fluorescence microscopy

Images were taken with a 40×/numerical aperture 1.25 plan apochromatic objective on a laser-scanning confocal microscope (SP5; Leica, Exton, PA). Contrast and relative intensities of the green (Alexa 488 or Venus tag), red (Alexa 594), and blue (DAPI) images were adjusted with Photoshop (Adobe, Mountain View, CA).

ACKNOWLEDGMENTS

We thank K. Praveen, Z. Lu, and T. K. Rajendra for advice and help, both technical and theoretical. We are indebted to L. Perkins, J. Gall, B. McCabe, and J. Szabad for reagents. This work was supported by National Institutes of Health Grant R01-GM053034 (to A.G.M.).

REFERENCES

Baccon J, Pellizzoni L, Rappsilber J, Mann M, Dreyfuss G (2002). Identification and characterization of Gemin7, a novel component of the survival of motor neuron complex. J Biol Chem 277, 31957–31962.

Baker SE, Lorenzen JA, Miller SW, Bunch TA, Jannuzi AL, Ginsberg MH, Perkins LA, Brower DL (2002). Genetic interaction between integrins and moleskin, a gene encoding a *Drosophila* homolog of importin-7. Genetics 162, 285–296.

Bhardwaj A, Cingolani G (2010). Conformational selection in the recognition of the snurportin importin beta binding domain by importin beta. Biochemistry 49, 5042–5047.

Bischof J, Maeda RK, Hediger M, Karch F, Basler K (2007). An optimized transgenesis system for *Drosophila* using germ-line-specific phiC31 integrases. Proc Natl Acad Sci USA 104, 3312–3317.

Bischoff FR, Klebe C, Kretschmer J, Wittinghofer A, Ponstingl H (1994). RanGAP1 induces GTPase activity of nuclear Ras-related Ran. Proc Natl Acad Sci USA 91, 2587–2591.

Brand A, Perrimon N (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development 118, 401–415.

Carissimi C, Baccon J, Straccia M, Chiarella P, Maiolica A, Sawyer A, Rappsilber J, Pellizzoni L (2005). Unrip is a component of SMN complexes active in snRNP assembly. FEBS Lett 579, 2348–2354.

Carissimi C, Saieva L, Baccon J, Chiarella P, Maiolica A, Sawyer A, Rappsilber J, Pellizzoni L (2006a). Gemin8 is a novel component of the survival motor neuron complex and functions in small nuclear ribonucleoprotein assembly. J Biol Chem 281, 8126–8134.

Carissimi C, Saieva L, Gabanella F, Pellizzoni L (2006b). Gemin8 is required for the architecture and function of the survival motor neuron complex. J Biol Chem 281, 37009–37016.

Chang HC *et al.* (2008). Modeling spinal muscular atrophy in *Drosophila*. PLoS One 3, e3209.

Dong X, Biswas A, Suel KE, Jackson LK, Martinez R, Gu H, Chook YM (2009). Structural basis for leucine-rich nuclear export signal recognition by CRM1. Nature 458, 1136–1141.

Erdélyi M, Máthé E, Szabad J (1997). Genetic and developmental analysis of mutant Ketel alleles that identify the *Drosophila* importin-beta homologue. Acta Biol Hung 48, 323–338.

Fischer U, Darzynkiewicz E, Tahara SM, Dathan NA, Lührmann R, Mattaj IW (1991). Diversity in the signals required for nuclear accumulation of U snRNPs and variety in the pathways of nuclear transport. J Cell Biol 113, 705–714.

Fischer U, Sumpter V, Sekine M, Satoh T, Lührmann R (1993). Nucleocytoplasmic transport of U snRNPs: definition of a nuclear location signal in the Sm core domain that binds a transport receptor independently of the m3G cap. EMBO J 12, 573–583.

Fischer U, Heinrich J, van Zee K, Fanning E, Lührmann R (1994). Nuclear transport of U1 snRNP in somatic cells: differences in signal requirement compared with *Xenopus laevis* oocytes. J Cell Biol 125, 971–980.

Freedman ND, Yamamoto KR (2004). Importin 7 and importin alpha/importin beta are nuclear import receptors for the glucocorticoid receptor. Mol Biol Cell 15, 2276–2286.

Görlich D, Dabrowski M, Bischoff FR, Kutay U, Bork P, Hartmann E, Prehn S, Izaurralde E (1997). A novel class of RanGTP binding proteins. J Cell Biol 138, 65–80.

Hamm J, Darzynkiewicz E, Tahara SM, Mattaj IW (1990). The trimethylguanosine cap structure of U1 snRNA is a component of a bipartite nuclear targeting signal. Cell 62, 569–577.

Hernandez N, Weiner AM (1986). Formation of the 3′ end of U1 snRNA requires compatible snRNA promoter elements. Cell 47, 249–258.

Huber J, Cronshagen U, Kadokura M, Marshallsay C, Wada T, Sekine M, Lührmann R (1998). Snurportin1, an m3G-cap-specific nuclear import receptor with a novel domain structure. EMBO J 17, 4114–4126.

Huber J, Dickmanns A, Lührmann R (2002). The importin-beta binding domain of snurportin1 is responsible for the Ran- and energy-independent nuclear import of spliceosomal U snRNPs in vitro. J Cell Biol 156, 467–479.

Jády BE, Darzacq X, Tucker KE, Matera AG, Bertrand E, Kiss T (2003). Modification of Sm small nuclear RNAs occurs in the nucleoplasmic Cajal body following import from the cytoplasm. EMBO J 22, 1878–1888.

Jäkel S, Albig W, Kutay U, Bischoff FR, Schwamborn K, Doenecke D, Görlich D (1999). The importin beta/importin 7 heterodimer is a functional nuclear import receptor for histone H1. EMBO J 18, 2411–2423.

Jäkel S, Görlich D (1998). Importin beta, transportin, RanBP5 and RanBP7 mediate nuclear import of ribosomal proteins in mammalian cells. EMBO J 17, 4491–4502.

James BP, Bunch TA, Krishnamoorthy S, Perkins LA, Brower DL (2007). Nuclear localization of the ERK MAP kinase mediated by *Drosophila* alphaPS2betaPS integrin and importin-7. Mol Biol Cell 18, 4190–4199.
Kubota T, Maezawa S, Koiwai K, Hayano T, Koiwai O (2007). Identification of functional domains in TdIF1 and its inhibitory mechanism for TdT activity. Genes Cells 12, 941–959.
Lemm I, Girard C, Kuhn AN, Watkins NJ, Schneider M, Bordonné R, Lührmann R (2006). Ongoing U snRNP biogenesis is required for the integrity of Cajal bodies. Mol Biol Cell 17, 3221–3231.
Lippai M *et al*. (2000). The Ketel gene encodes a *Drosophila* homologue of importin-beta. Genetics 156, 1889–1900.
Liu J, Hebert MD, Ye Y, Templeton DJ, Kung H, Matera AG (2000). Cell cycle-dependent localization of the CDK2-cyclin E complex in Cajal (coiled) bodies. J Cell Sci 113, 1543–1552.
Liu JL, Buszczak M, Gall JG (2006a). Nuclear bodies in the *Drosophila* geminal vesicle. Chromosome Res 14, 465–475.
Liu JL, Murphy C, Buszczak M, Clatterbuck S, Goodman R, Gall JG (2006b). The *Drosophila melanogaster* Cajal body. J Cell Biol 172, 875–884.
Liu JL, Gall JG (2007). U bodies are cytoplasmic structures that contain uridine-rich small nuclear ribonucleoproteins and associate with P bodies. Proc Natl Acad Sci USA 104, 11655–11659.
Lorenzen JA,, Baker SE, Denhez F, Melnick MB, Brower DL, Perkins LA (2001). Nuclear import of activated D-ERK by DIM-7, an importin family member encoded by the gene moleskin. Development 128, 1403–1414.
Marshallsay C, Lührmann R (1994). In vitro nuclear import of snRNPs: cytosolic factors mediate m3G-cap dependence of U1 and U2 snRNP transport. EMBO J 13, 222–231.
Matera AG, Terns RM, Terns MP (2007). Non-coding RNAs: lessons from the small nuclear and small nucleolar RNAs. Nat Rev Mol Cell Biol 8, 209–220.
Mattaj IW, De Robertis EM (1985). Nuclear segregation of U2 snRNA requires binding of specific snRNP proteins. Cell 40, 111–118.
Meister G, Eggert C, Fischer U (2002). SMN-mediated assembly of RNPs: a complex story. Trends Cell Biol 12, 472–478.
Mitrousis G, Olia AS, Walker-Kopp N, Cingolani G (2008). Molecular basis for the recognition of snurportin 1 by importin beta. J Biol Chem 283, 7877–7884.
Monecke T, Guttler T, Neumann P, Dickmanns A, Gorlich D, Ficner R (2009). Crystal structure of the nuclear export receptor CRM1 in complex with Snurportin1 and RanGTP. Science 324, 1087–1091.
Mouaikel J, Verheggen C, Bertrand E, Tazi J, Bordonné R (2002). Hypermethylation of the cap structure of both yeast snRNAs and snoRNAs requires a conserved methyltransferase that is localized to the nucleolus. Mol Cell 9, 891–901.
Narayanan U, Ospina JK, Frey MR, Hebert MD, Matera AG (2002). SMN, the spinal muscular atrophy protein, forms a pre-import snRNP complex with snurportin1 and importin beta. Hum Mol Genet 11, 1785–1795.
Narayanan U, Achsel T, Lührmann R, Matera AG (2004). Coupled in vitro import of U snRNPs and SMN, the spinal muscular atrophy protein. Mol Cell 16, 223–234.
Ohno M, Segref A, Bachi A, Wilm M, Mattaj IW (2000). PHAX, a mediator of U snRNA nuclear export whose activity is regulated by phosphorylation. Cell 101, 187–198.
Ospina JK, Gonsalvez GB, Bednenko J, Darzynkiewicz E, Gerace L, Matera AG (2005). Cross-talk between snurportin1 subdomains. Mol Biol Cell 16, 4660–4671.
Palacios I, Hetzer M, Adam SA, Mattaj IW (1997). Nuclear import of U snRNPs requires importin beta. EMBO J 16, 6783–6792.
Paraskeva E, Izaurralde E, Bischoff FR, Huber J, Kutay U, Hartmann E, Lührmann R, Görlich D (1999). CRM1-mediated recycling of snurportin 1 to the cytoplasm. J Cell Biol 145, 255–264.
Pellizzoni L, Yong J, Dreyfuss G (2002). Essential role for the SMN complex in the specificity of snRNP assembly. Science 298, 1775–1779.
Praveen K, Wen Y, Matera AG (2012). A *Drosophila* model of spinal muscular atrophy uncouples snRNP biogenesis functions of survival motor neuron from locomotion and viability defects. Cell Rep 1, 624–631.
Sauterer RA, Feeney RJ, Zieve GW (1988). Cytoplasmic assembly of snRNP particles from stored proteins and newly transcribed snRNA's in L929 mouse fibroblasts. Exp Cell Res 176, 344–359.
Shpargel KB, Matera AG (2005). Gemin proteins are required for efficient assembly of Sm-class ribonucleoproteins. Proc Natl Acad Sci USA 102, 17372–17377.
Sleeman JE, Lamond AI (1999). Newly assembled snRNPs associate with coiled bodies before speckles, suggesting a nuclear snRNP maturation pathway. Curr Biol 9, 1065–1074.
Strasser A, Dickmanns A, Lührmann R, Ficner R (2005). Structural basis for m3G-cap-mediated nuclear import of spliceosomal snRNPs by snurportin 1. EMBO J 24, 2235–2243.
Tanackovic G, Kramer A (2005). Human splicing factor SF3a, but not SF1, is essential for pre-mRNA splicing in vivo. Mol Biol Cell 16, 1366–1377.
Villányi Z, Debec A, Timinszky G, Tirián L, Szabad J (2008). Long persistence of importin-beta explains extended survival of cells and zygotes that lack the encoding gene. Mech Dev 125, 196–206.
Wang J, Beck E, McCabe B (2012). A modular toolset for recombination transgenesis and neurogenetic analysis of *Drosophila*. PLoS One 7, e42102.
Wersig C, Guddat U, Pieler T, Bindereif A (1992). Assembly and nuclear transport of the U4 and U4/U6 snRNPs. Exp Cell Res 199, 373–377.
Yong J, Golembe TJ, Battle DJ, Pellizzoni L, Dreyfuss G (2004). snRNAs contain specific SMN-binding domains that are essential for snRNP assembly. Mol Cell Biol 24, 2747–2756.