Chromosoma
DOI 10.1007/s00412-006-0056-6

REVIEW

David Staněk · Karla M. Neugebauer

The Cajal body: a meeting place for spliceosomal snRNPs in the nuclear maze

Received: 7 December 2005 / Revised: 27 January 2006 / Accepted: 29 January 2006

Abstract Spliceosomal small nuclear ribonucleoprotein particles (snRNPs) are essential pre-mRNA splicing factors that consist of small nuclear RNAs (snRNAs) complexed with specific sets of proteins. A considerable body of evidence has established that snRNP assembly is accomplished after snRNA synthesis in the nucleus through a series of steps involving cytoplasmic and nuclear phases. Recent work indicates that snRNPs transiently localize to the Cajal body (CB), a nonmembrane-bound inclusion present in the nuclei of most cells, for the final steps in snRNP maturation, including snRNA base modification, U4/U6 snRNA annealing, and snRNA-protein assembly. Here, we review these findings that suggest a crucial role for CBs in the spliceosome cycle in which production of new snRNPs—and perhaps regenerated snRNPs after splicing—is promoted by the concentration of substrates in this previously mysterious subnuclear organelle. These insights allow us to speculate on the role of nuclear bodies in regulating the dynamics of RNP assembly to maintain a functional pool of factors available for key steps in gene expression.

Communicated by G. Matera

D. Staněk (✉)
Department of Cellular Biology and Pathology,
First Medical Faculty,
Charles University and Institute of Physiology,
Academy of Sciences of the Czech Republic,
Albertov 4,
Prague 2, 128 00, Czech Republic
e-mail: dstan@lf1.cuni.cz
Tel.: +420-2-24968015
Fax: +420-2-24917418

K. M. Neugebauer
Max Planck Institute of Molecular Cell Biology and Genetics,
Pfotenhauerstrasse 108,
01307 Dresden, Germany

Introduction

Pre-mRNA splicing is accomplished by the spliceosome, a macromolecular machine composed of hundreds of proteins and five small nuclear RNAs (snRNAs), which resembles the ribosome in size and complexity (Jurica and Moore 2003). In contrast to the ribosome, the spliceosome is assembled from preformed snRNA-containing particles [small nuclear ribonucleoprotein particles (snRNPs)] and a host of non-snRNP proteins. Spliceosomal snRNPs consist of one or two snRNAs for which each is named, along with several associated proteins. SnRNP assembly is a complicated process that begins in the nucleus, continues in the cytoplasm, and finishes again in the nucleus. Our task in this review is to overlay what is known from in vitro studies about the multiple steps in snRNP assembly pathways with the three-dimensional space of the cell nucleus. We will summarize recent progress in the field that indicates that Cajal bodies (CBs), subnuclear structures in which snRNPs are concentrated (Fig. 1), are the sites of particular steps in spliceosomal snRNP assembly.

CBs are nonmembrane-bound nuclear bodies, 0.5–1.0 μm in diameter, present within the nuclei of most cells (Gall 2000). They were first observed by the Spanish neurobiologist Ramón y Cajal who named them accessory bodies (Cajal 1903). The bodies were then forgotten and rediscovered by electron microscopists and, according to their appearance in the electron microscope, called coiled bodies (Monneron and Bernhard 1969). Interest in Cajal bodies rose when a marker protein, coilin, was identified and cloned (Andrade et al. 1991; Raska et al. 1991; Tuma et al. 1993), enabling researchers to identify many additional molecular constituents of CBs and fueling speculation as to the function of this mysterious nuclear body (Matera 1999). In recognition of Cajal's discovery and to unify nomenclature, the bodies were renamed after Cajal (Gall 2000).

Cajal bodies are dynamic structures within the nucleus (reviewed in Carmo-Fonseca et al. 2002; Cioce and Lamond 2005). They move, split, and rejoin in the nucleoplasm and their size and number depends on cell metabolic

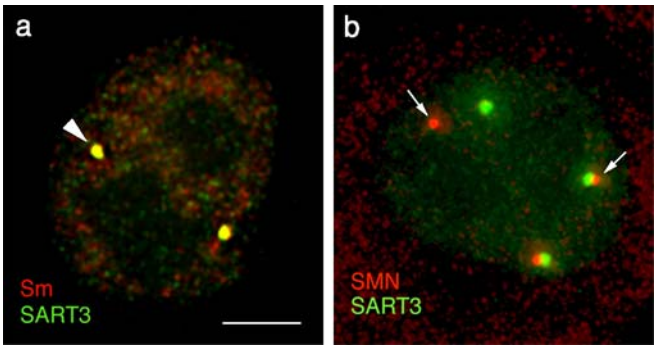

Fig. 1 Localization of snRNPs, SART3, and SMN in HeLa-KN cell nucleus. **a** SART3 and snRNPs are distributed throughout the nucleoplasm and they colocalize in Cajal bodies (*arrowhead*). **b** SART3 accumulates in Cajal bodies and not in gems. SART3 concentrates in CBs while SMN is localized in the cytoplasm and in the cell nucleus concentrated in gems (*arrows*). HeLa-KN is a variant HeLa cell line with low methyltransferase activity; this correlates with coilin hypomethylation and separation of gems and CBs (Hebert et al. 2002). SnRNPs were visualized using the Y12 antibody recognizing Sm proteins, SART3 using polyclonal antibodies, and SMN using the monoclonal antibody 2B1. *Bar* 5 μm

activity and the cell cycle (Andrade et al. 1993; Boudonck et al. 1998, 1999; Carmo-Fonseca et al. 1993; Carvalho et al. 1999; Platani et al. 2002, 2000). Like other nuclear structures, CBs exchange their molecular contents with surrounding nucleoplasm and exchange rates vary greatly among different proteins; even coilin exchanges completely with the nucleoplasmic pool within minutes (Dundr et al. 2004; Handwerger et al. 2003; Sleeman et al. 2003). These features suggest that molecular functions residing within CBs are in equilibrium with surrounding nucleoplasm. It is currently thought that factors traffic to CBs where they function in metabolism of different classes of RNP particles (e.g., spliceosomal snRNPs, small nucleolar RNPs, telomerase, and U7 snRNP) and in histone mRNA 3′-end processing (reviewed in Carmo-Fonseca 2002; Cioce and Lamond 2005; Gall 2000; Jady et al. 2005; Kiss et al. 2002; Kiss 2004; Ogg and Lamond 2002; Schumperli and Pillai 2004). Here, we focus on recent insights into the specific steps in spliceosomal snRNP assembly that take place in CBs and consider that similar principles may apply to other proposed CB functions.

The "life cycle" of the snRNAs is schematized in Fig. 2. With the exception of U6 snRNA transcribed by RNA polymerase III, all spliceosomal snRNAs (U1, U2, U4, and U5) are transcribed by RNA polymerase II and receive a 7-methyl-guanosine cap at their 5′ end cotransciptionally. Primary processing at the 3′-end likely occurs at the site of transcription because 3′-end processing is regulated by snRNA-specific promoters and phosphorylation of the C-terminal domain (CTD) of the RNA polymerase II (Jacobs et al. 2004; Medlin et al. 2003; Uguen and Murphy 2003). Recently, a new complex named the Integrator was shown to bind the CTD and to be necessary for U1 and U2 pre-snRNA cleavage at the 3′-end (Baillat et al. 2005). Because pre-snRNAs themselves and snRNA transcription factors are detectable in CBs, it was suggested that CBs function in pre-snRNA processing and/or transcriptional regulation of snRNA genes (Matera 1998; Schul et al. 1998; Smith and Lawrence 2000). Moreover, a significant proportion of CBs lie adjacent to snRNA genes and transcriptional activity of the U1 and U2 genes and snRNA coding region are required for CB association (Frey et al. 1999; Frey and Matera 2001; Jacobs et al. 1999; Platani et al. 2002; Smith et al. 1995).

Newly synthesized snRNAs are exported to the cytoplasm due to their interaction with PHAX and the cap-binding complex (Ohno et al. 2000; Segref et al. 2001). The observation that PHAX is concentrated in CBs (Massenet et al. 2002) is consistent with the possibility that newly transcribed snRNAs are loaded cotranscriptionally with PHAX in CBs. Alternatively (or in addition), PHAX may be present in CBs due to its distinct role in small nucleolar RNA (snoRNA) trafficking to the CB (Boulon et al. 2004; Watkins et al. 2004). In the cytoplasm, a core snRNP is formed by the ATP-dependent assembly of a ring of seven Sm proteins on each snRNA. The survival motor neuron (SMN) complex plays a crucial role in the fidelity of this process (reviewed in Meister et al. 2002; Paushkin et al. 2002; Terns and Terns 2001). Assembly of the Sm ring is followed by cytoplasmic hypermethylation of the 5′ cap yielding 2,2,7-tri-methyl-guanosine. The tri-methyl cap, together with the Sm ring, serves as a nuclear import signal. The SMN complex, which stays associated with snRNPs throughout the whole cytoplasmic phase (Massenet et al. 2002), interacts with snurportin1 and facilitates nuclear import of snRNPs (Narayanan et al. 2002, 2004). It is interesting to note that a fraction of SMN complex components is nuclear. In most cell lines, nuclear SMN localizes to CBs and CB localization is regulated by methylation of coilin (Hebert et al. 2002). In cell lines with low methyltransferase activity, SMN concentrates in CB-related structures called gems (Fig. 1 shows gems in HeLa-KN strain with low methyltransferase activity; see Hebert et al. 2002 for details). The nuclear localization of the SMN complex suggests that SMN plays a role in snRNP targeting to CBs (see below) and/or is simply deposited in CBs after import. Nuclear SMN may also play additional roles, as yet undefined, in Sm or Like-Sm (LSm) ring assembly and/or pre-mRNA splicing (Friesen et al. 2001; Pellizzoni et al. 1998). Recently, the unrip and ZPR1 proteins were identified as new members of the SMN complex that influence SMN distribution between the cytoplasm and the nucleus (Carissimi et al. 2005; Grimmler et al. 2005) and its localization to CBs (Gangwani et al. 2005).

Once back in the cell nucleus, snRNAs are modified by 2′-*O*-ribose methylation and pseudouridylation (Jady et al. 2003; Kiss 2004). It is also believed that most snRNP-specific proteins are added to the core snRNPs in the cell nucleus (see below). The snRNPs imported from the cytoplasm first appear in CBs, suggesting that final maturation steps occur in CBs before new snRNPs can participate in pre-mRNA splicing, which takes place cotranscriptionally throughout the nucleoplasm (Neugebauer 2002; Sleeman et

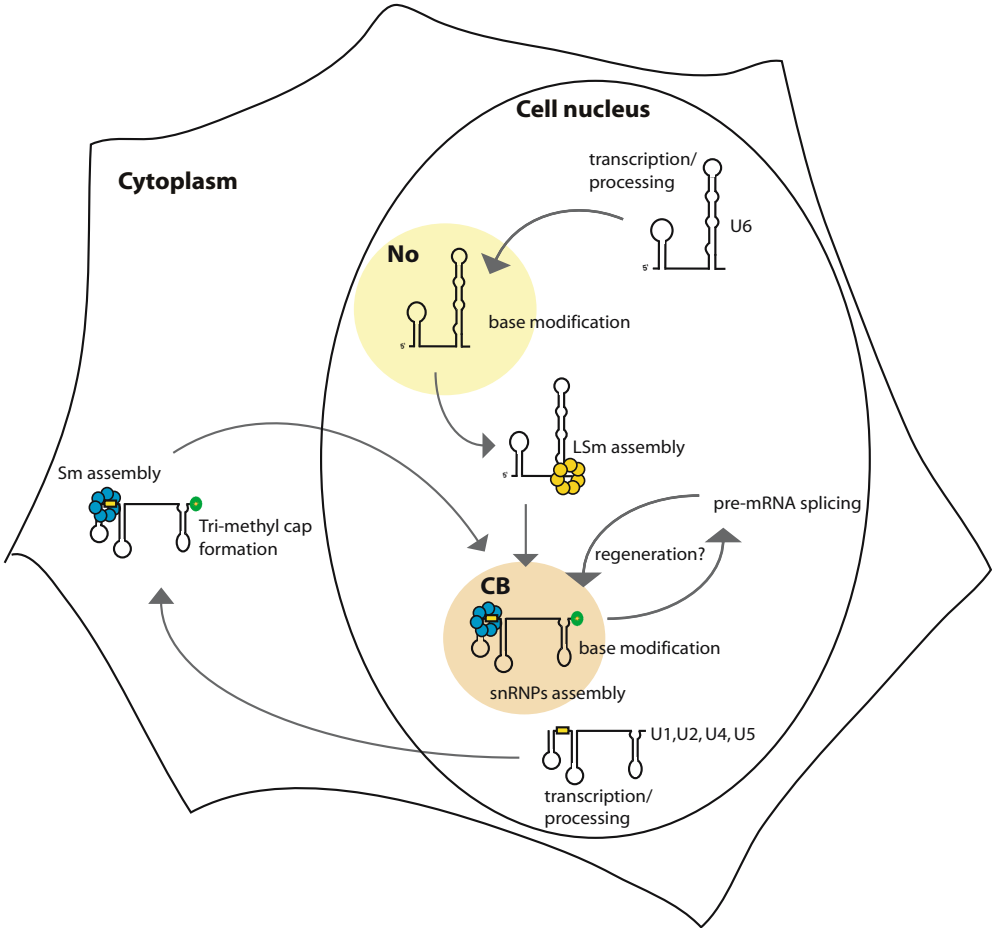

Fig. 2 Biogenesis of the spliceosomal snRNPs. U1, U2, U4, and U5 snRNAs are transcribed in the cell nucleus by RNA polymerase II, which regulates their processing as well. They are then exported to the cytoplasm where the Sm ring (*blue balls*) is assembled and hypermethylation of the 5′ end occurs. The Sm ring and the trimethyl cap (*green ball*) act as signals for nuclear reimport. Back in the nucleus, U snRNAs first visit Cajal bodies (*CB*, *beige compartment*) where specific nucleotides are modified by base methylation and pseudouridylation and where assembly of the U2, U4/U6, and U4/U6•U5 snRNPs occurs. The U6 snRNA is transcribed by RNA polymerase III, receives a γ monomethyl cap and is thought to remain in the nucleus. The U6 snRNA is base modified in the nucleolus (*No*, *yellow compartment*) and then targeted to Cajal bodies where it is incorporated into U4/U6 and U4/U6•U5 snRNPs. Assembly of the LSm ring (*yellow balls*) on U6 is likely nucleoplasmic. Assembled U4/U6•U5 snRNPs leave the Cajal body and function in splicing in the nucleoplasm; individual snRNPs disassembled by the splicing process potentially return to Cajal bodies for regeneration of active complexes

al. 2001; Sleeman and Lamond 1999). Indeed, Cajal body-specific RNAs (scaRNAs) that guide snRNA modifications are localized in CBs and snRNA targeting to CBs is necessary for proper posttranscriptional nucleotide modification (Darzacq et al. 2002; Jady et al. 2003; Kiss et al. 2002; Kiss 2004). ScaRNAs containing the H/ACA motif are targeted to CBs via special Cajal body-specific localization signal, the Cajal body box (CAB box), which resides in the H/ACA motif (Richard et al. 2003). The mechanism of CAB box targeting to CBs is currently unknown.

U1 snRNP

Besides the U1 snRNA and common Sm proteins, the human U1 snRNP contains three specific proteins: U1-70K, U1-A, and U1-C (Table 1). The two largest U1-specific proteins, U1-70K and U1-A, bind directly to the U1 snRNA while the U1-C protein is attached through protein–protein interactions with U1-70K and Sm proteins (Nelissen et al. 1994). U1-A and U1-C are imported to the nucleus independently of the U1 snRNA (Jantsch and Gall 1992; Kambach and Mattaj 1992), indicating that U1 snRNP assembly occurs in the nucleus (Feeney and Zieve 1990). The role of CBs in this process is not clear. In contrast to other snRNAs, the U1 snRNA is not abundant in CBs (Carmo-Fonseca et al. 1992; Matera and Ward 1993) and so far, only one U1-specific protein, the U1-C protein, was detected in CBs (Hoet et al. 1998).

U2 snRNP

The active 17S U2 snRNP comprises the U2 snRNAs, the core Sm proteins, two U2-specific proteins U2A′ and U2B″,

Table 1 Spliceosomal snRNP proteins

U1 snRNP
Sm proteins (B/B′, D1–3, E, F, G)
U1-70K
U1-A
U1-C
U2 snRNP
Sm proteins (B/B′, D1–3, E, F, G)
U2-A′
U2-B″
SF3a complex
SF3a120
SF3a66
SF3a60
SF3b complex
SF3b155
SF3b145
SF3b130
SF3b125[a]
SF3b49
SF3b14b
SF3b10
p14
17S associated proteins
SPF30
hPrp5
SR140
CHERP
hPrp43
Hsp75
PUF60
U2AF35
U2AF65
Hsp60
SPF45
BRAF35
SPF31
SF2/AS
20S U5 snRNP
Sm proteins (B/B′, D1–3, E, F, G)
hDib/15K
U5-40K
hPrp28/100K
hPrp6/102K
hSnu114/116K
hBrr2/200K
hPrp8/220K
52K/CD2BP2[b]
U4/U6 snRNP
Sm proteins (B/B′, D1–3, E, F, G)
LSm proteins (2–8)
hPrp24/SART3/p110[b]
hSnu13/15.5K/NHPX
hPrp31/61K
hPrp3/90K
hPrp4/60K
USA-Cyp/20K
U4/U6•U5 snRNP specific
27K
hSad1/65K
hSnu66/SART1/110K

[a]Not present in the 17S U2 snRNP
[b]Not present in the U4/U6•U5 tri-snRNP

and many other associated proteins (see Table 1 and Will et al. 2002). In vitro formation of the mature 17S U2 snRNP proceeds in a stepwise manner. First, the 12S U2 snRNP, which consists of the U2 snRNA, Sm proteins, U2B″, and U2A′ interacts with the SF3b complex and forms an intermediate 15S particle. The 17S particle is then formed by association of the 15S particle with the complex SF3a (Brosi et al. 1993; Kramer et al. 1999). In *Saccharomyces cerevisiae*, Cus2p promotes assembly of the 17S U2 snRNP and the human homologue Tat-SF1 was suggested to function in a similar manner (Yan et al. 1998).

Several lines of evidence indicate that assembly of the 12S and 17S particles occurs in the cell nucleus after reimport of the newly assembled core U2 snRNP from the cytoplasm. First, U2A′ and U2B″ are imported to the nucleus independently of the U2 snRNA (Kambach and Mattaj 1994). Second, in *Xenopus laevis* oocyte, U2 snRNA modifications, which occur in the nucleus after reimport from the cytoplasm, are required for assembly of the 17S U2 snRNP (Yu et al. 1998; Zhao and Yu 2004). Because U2 snRNA modifications occur in CBs and the U2 snRNA, U2A′ and U2B″ are located to CBs as well, the assembly of the 12S particle likely takes place in this nuclear structure. Recently, CBs were directly implicated in further steps of U2 snRNP assembly (Nesic et al. 2004). Mutant forms of SF3a60 and SF3a66, which are unable to incorporate into the 17S U2 snRNP, accumulate in CBs (Nesic and Kramer 2001; Nesic et al. 2004). SF3b125, which was found in isolated SF3b complexes but lacking in mature 17S U2 snRNPs, was implicated in 17S maturation and detected in CBs as well (Will et al. 2002). However, wild-type SF3a proteins and several other proteins found in the 17S particle were not detected in CBs. It is possible that formation of both 12S and 17S U2 snRNPs take place in CBs; rapid release of the mature 17S particle from the CB may explain why 17S-specific proteins escape detection.

U5 snRNP

The U5 snRNA is detectable in three distinct complexes within nuclear extracts: the 20S U5 snRNP, the 25S U4/U6•U5 tri-snRNP, and the 35S U5 snRNP (Makarov et al. 2002). The 35S snRNP likely represents a post-spliceosomal form of the U5 snRNP with proteins of the Prp19 complex (Makarov et al. 2002). The abundant 20S complex is believed to interact with the U4/U6 snRNP to yield the U4/U6•U5 tri-snRNP, which is competent for spliceosome assembly. The 20S U5 snRNP contains, in addition

to a set of Sm proteins, at least eight additional proteins (see Table 1) (Behrens and Luhrmann 1991; Laggerbauer et al. 2005) but nothing is known about its formation.

U4/U6 snRNP

The composition of the U4 snRNP remains elusive because it has been impossible to biochemically isolate the mono-U4 snRNP. However, protein components of the U4/U6 snRNP in which the U4 and U6 snRNAs are annealed by base pairing are known (see Table 1). Binding of the 15.5 kD protein to the U4 snRNA (Watkins et al. 2000) is necessary for recruitment of hPrp31 to the U4 snRNA (Nottrott et al. 2002). In contrast, the hPrp3/hPrp4/USA-CyP complex binds U4/U6 RNA duplex (Nottrott et al. 2002), indicating that this complex associates after U4/U6 snRNA annealing. The hPrp3/hPrp4/USA-CyP complex can form even in the absence of RNA (Horowitz et al. 1997; Teigelkamp et al. 1998) and it is currently unclear whether it stays associated with the U4 snRNP after splicing.

The U6 snRNA differs from the rest of the snRNAs in many ways. It is the only spliceosomal snRNA transcribed by RNA polymerase III; as a result, it does not receive a 7-methyl guanosine cap but rather undergoes methylation of the guanosine gamma phosphate at the 5′-end (Singh and Reddy 1989). Newly synthesized U6 snRNA does not leave the cell nucleus during its maturation but is bound at its 3′-end by the La protein, which stabilizes the new transcript. U6 snRNA base modifications are guided by snoRNPs, which reside in the nucleolus (Ganot et al. 1999; Tycowski et al. 1998). Consistent with this finding, the U6 snRNA transiently localizes in the nucleolus of *Xenopus* oocyte (Gerbi et al. 2003; Lange and Gerbi 2000). The La protein is replaced by seven LSm proteins (2–8), which form a similar ring as Sm proteins and promote annealing of U4/U6 in vitro (Achsel et al. 1999). The LSm proteins can be transported to the nucleus as a preformed complex, which further supports the nuclear model of U6 snRNP maturation (Zaric et al. 2005); it is currently unknown whether SMN plays a role in LSm ring assembly on U6, but the fact that SMN binds at least a subset of LSm proteins suggests that it may (Friesen et al. 2001). So far, the only other U6-specific protein identified is SART3 (also named hPrp24 or p110), which is required for assembly of U4/U6 snRNPs in vitro (Bell et al. 2002; Rader and Guthrie 2002). SART3 contains two RNA-recognition motifs that bind directly to the U6 snRNA (Bell et al. 2002; Medenbach et al. 2004). In addition, SART3 interacts via its C terminal domain with LSm proteins and through its N-terminal HAT domain with the U4/U6-specific protein hPrp3 (Medenbach et al. 2004; Rader and Guthrie 2002). These complex interactions suggest that SART3 may provide the scaffolding for U4/U6 snRNP formation. Moreover, SART3 must leave U4/U6 snRNPs as the tri-snRNP is being formed (see Fig. 3) because it is not detected in the U4/U6•U5 tri-snRNP or the spliceosome (Bell et al. 2002).

Where is the U4/U6 snRNP formed? Three lines of evidence support the conclusion that U4/U6 snRNP assembly occurs in CBs (see Figs. 1 and 3). First, SART3, U4 and U6 snRNAs, and U6- and U4/U6-specific proteins are enriched in CBs (Carmo-Fonseca et al. 1992; Makarova et al. 2002; Matera and Ward 1993; Schaffert et al. 2004; Stanek and Neugebauer 2004; Stanek et al. 2003). It is important to note that U4 and U6 mono-snRNPs are targeted independently to CBs (Stanek and Neugebauer 2004; Stanek et al. 2003), indicating that the mono-snRNP components and not the preformed U4/U6 snRNP traffic to CBs. Second, by fluorescence resonance energy transfer (FRET) analysis, transient intermediates containing SART3 and U4/U6-specific proteins hPrp31, hPrp3, and hPrp4 are highly enriched in CBs (Stanek and Neugebauer 2004). The transition to the U4/U6 snRNP in the CB is likely accompanied by a rapid conformational change because FRET studies revealed that SART3 interacts with LSm proteins (indicative of the U6 snRNP) in the nucleoplasm but not in CBs (Stanek and Neugebauer 2004). Third, inhibition of the next step in the assembly pathway, formation of the U4/U6•U5 tri-snRNP, leads to accumulation of the U4/U6 snRNP in CBs (Schaffert et al. 2004; see below).

U4/U6•U5 tri-snRNP

Tri-snRNP formation follows U4/U6 snRNP assembly by addition of the 20S U5 snRNP through protein–protein interactions. Two proteins, U5-specific hPrp6 and the U4/U6 snRNP-specific hPrp31, interact with each other and are necessary for tri-snRNP formation (Makarova et al. 2002; Schaffert et al. 2004). Knockdown of either of these proteins by small-interfering RNA in tissue culture cells leads to depletion of the tri-snRNP in cells (Schaffert et al. 2004). Recently, the U5-52K protein was identified as a specific component of the 20S U5 snRNP; the 52K protein directly interacts with hPrp6 but is not present in the tri-snRNP, suggesting that it is involved in tri-snRNP formation (Laggerbauer et al. 2005).

Inhibition of tri-snRNP formation by knockdown of hPrp6 or hPrp31 leads to accumulation of the U4/U6 snRNPs in CBs, while levels of the U5 snRNA and U5-specific proteins remain unchanged (Schaffert et al. 2004). The simplest explanation for these data is that U4 and U6 snRNAs are annealed in CBs (see above) and the resulting U4/U6 snRNP is retained there until the U5 snRNP joins; only when the mature tri-snRNP is formed is it released from the CB (see Fig. 3). Consistent with this, SART3 residence time in CBs is shorter than Sm proteins (Dundr et al. 2004) and 20S-specific U5-52K does not accumulate in CBs (Laggerbauer et al. 2005). Taken together, the evidence suggests that tri-snRNP assembly in CBs is relatively fast and raises the possibility that snRNP assembly in

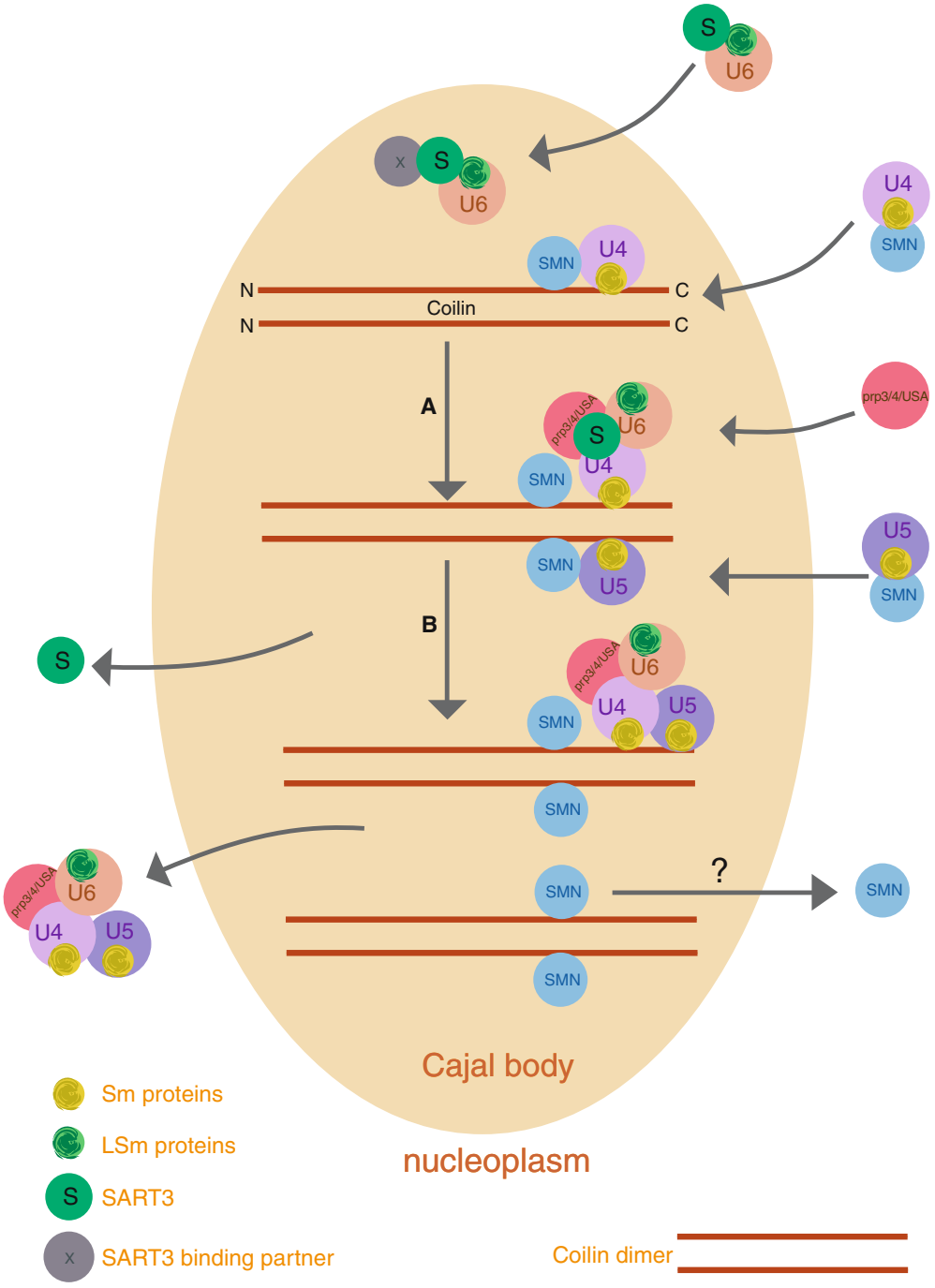

Fig. 3 Diagram showing the various steps in U4/U6 and U4/U6•U5 snRNP assembly in Cajal bodies including a speculative model for how the individual snRNPs might interact with coilin during the assembly process. Coilin is drawn as a dimer oriented with N termini aligned, based on in vitro binding assays and in situ FRET measurements (Hebert and Matera 2000; Stanek and Neugebauer 2004). The U6 snRNP is targeted to the CB by SART3, which interacts via its HAT domain with a hypothetical binding partner X. The U4 and U5 snRNPs are shown entering the CB with SMN, which interacts with the *RG box* near the Sm binding site at the C terminus of coilin (Frey and Matera 1995; Xu et al. 2005). Upon Sm and SMN binding to coilin, the U4 and U5 snRNPs are "handed off" to coilin as suggested by Tucker and Matera (2005). In step A, U4 and U6 snRNAs are annealed by SART3 and LSm proteins; a conformational change is shown in which SART3 moves away from U6's LSm ring and interacts instead with U4/U6 proteins (Stanek and Neugebauer 2004). In the same step, the hPrp3/hPrp4/USA-CyP complex is recruited to the U4/U6 duplex (Nottrott et al. 2002) and the U4/U6 snRNP is complete. In step B, the U5 snRNP bound to a nearby coilin molecule joins the U4/U6 snRNP through protein–protein interactions (Makarova et al. 2002; Schaffert et al. 2004). At the same time, SART3 is lost from the complex and leaves the CB, accounting for its short residence time in CBs (Dundr et al. 2004). The mature U4/U6•U5 tri-snRNP is released from the CB and into the nucleoplasm where splicing occurs. SMN exchanges with nucleoplasm, permitting new snRNPs to bind coilin for subsequent rounds of assembly

CBs occurs more rapidly than it does in the nucleoplasm (see below). Unfortunately, the dynamics of further specific snRNP proteins as they traffic to and from CBs is yet been examined.

SnRNP regeneration after splicing

During splicing, snRNPs undergo extensive rearrangements (Staley and Guthrie 1998). The most dramatic changes occur to the U4/U6 snRNP and the U4/U6•U5 tri-snRNP. The U5 and U6 snRNAs play a central role in splicing catalysis, while the U4 snRNA dissociates from the spliceosome before or coincident with spliceosome activation (Chan et al. 2003; Jurica et al. 2002; Makarov et al. 2002; Makarova et al. 2004). This suggests that the U4 snRNA serves as a chaperone for U6. Thus, splicing destroys the U4/U6 snRNP and U4 and U6 snRNAs have to be reannealed and the snRNP has to be reassembled before they can participate in the next round of splicing. SART3 and LSm proteins promote annealing of U4 and U6 in vitro (Achsel et al. 1999; Bell et al. 2002) but their function in U4/U6 snRNP regeneration in vivo has yet to be addressed. The fact that SART3 and snRNPs are depleted from CBs upon inhibition of transcription and splicing suggests that disassembled postsplicing snRNPs indeed traffic to CBs for reassembly (Carmo-Fonseca et al. 1992; Stanek et al. 2003). However, contrary to the regeneration model, CBs disintegrate after inhibition of new snRNP import by leptomycin B or by depletion of SMN (Carvalho et al. 1999; Shpargel and Matera 2005); it is not clear whether these effects are direct or indirect. Therefore, further experiments are needed to provide direct evidence for snRNP recycling through CBs.

Similar to the U4/U6 snRNP, the tri-snRNP rearranges during splicing and regeneration of the active tri-snRNP is necessary. The postspliceosomal 35S U5 snRNP must be recycled and the 20S U5 snRNP regenerated, but so far no data are available on this step. Accumulation of the U4/U6 snRNP in CBs after inhibition of tri-snRNP formation by knockdown of hPrp6 or hPrp31 led Lührmann and colleagues to propose that tri-snRNP regeneration after splicing occurs in CBs (Schaffert et al. 2004). Because proteins found in the postspliceosomal 35S U5-snRNP particle are not detected in CBs, 35S U5 snRNP disassembly does not likely take place in CBs (M. Nousch, D. S., and K. N., unpublished data). This also indicates that if CBs are the sites of tri-snRNP regeneration after splicing, U4, U6, and U5 snRNPs individually enter the CB from surrounding nucleoplasm.

It is presently unclear whether U1 and U2 snRNPs require reassembly before splicing anew. U1 snRNP remodeling has not been reported. However, some proteins detected in the 17S U2 snRNP particle are not found in the activated spliceosome (Makarov et al. 2002), indicating that some regeneration of the U2 snRNP after splicing may be necessary.

Minor snRNPs

The minor spliceosomal snRNPs, which recognize introns bounded by AT at the 5′ splice site (instead of the canonical GU) and AC at the 3′ splice site (instead of AG), represent only 1% of the total spliceosomal snRNPs, reflecting the relatively small number (~500 in human) of "ATAC" introns (Will and Luhrmann 2005). Little is known about how these minor particles assemble. The U11/U12 snRNP is a composite snRNP that substitutes for the U1 and U2 snRNPs; it shares many proteins with U2 snRNP and lacks classical U1-specific proteins. Instead, it contains a set of new proteins specifically associated with the U11 snRNA (Will et al. 2004). There are no data available about localization of these proteins.

The atacU4/atacU6 snRNP and the atacU4/atacU6•U5 snRNP have identical protein composition to their major counterparts and it is likely that their assembly follows the same pathway as the major snRNPs (Schneider et al. 2002). Indeed, SART3 was shown to be necessary for regeneration of the atacU4/atacU6 snRNP in vitro, but in contrast to the U4/U6 snRNP, SART3 is not a component of the atacU4/atacU6 snRNP (Damianov et al. 2004). Moreover, hPrp31 is also important for assembly of the atacU4/atacU6•U5 snRNP (Schneider et al. 2002). Based on the information obtained on the major spliceosomal snRNPs, it is conceivable that minor snRNPs are assembled in CBs.

Targeting of spliceosomal snRNPs to CBs

So far, no "permanent" component of CBs was identified. Every CB protein tested exchanges with nucleoplasm, although the rates differ greatly. Coilin and SMN reside in CBs longer than any other proteins tested (Deryusheva and Gall 2004; Dundr et al. 2004; Handwerger et al. 2003; Sleeman et al. 2003). It was suggested that CBs form and are held together through relatively transient protein–protein interactions (Lewis and Tollervey 2000; Misteli 2001). RNP movements within the cell nucleus appear to be undirected (Molenaar et al. 2004; Shav-Tal et al. 2004); thus, we anticipate that snRNP movement through the nucleus is driven mostly by diffusion. Once they hit CBs, snRNPs must be retained there through interactions with proteins enriched in CBs.

The SMN complex, which is necessary for snRNP maturation in the cytoplasm, is also important for snRNP import to the cell nucleus (Narayanan et al. 2002, 2004). SMN interacts directly with coilin and this interaction may provide a targeting signal for newly imported snRNPs (Hebert et al. 2001, 2002). Furthermore, coilin binds Sm proteins (Xu et al. 2005) and apparently retains snRNPs in CBs because cells lacking functional coilin fail to accumulate snRNPs in nuclear bodies, even though SMN continues to be concentrated in gems in the absence of coilin (Tucker et al. 2001). These observations are consistent with a model (Fig. 3) in which SMN "hands

off' newly imported snRNPs to coilin in CBs (Tucker and Matera 2005).

The U6 snRNP does not contain Sm proteins but is also detected in CBs of mammalian cells (Carmo-Fonseca et al. 1992; Matera and Ward 1993; Schaffert et al. 2004; Stanek et al. 2003). Instead, the U6 snRNP is targeted to CBs through the HAT domain of SART3 (Stanek et al. 2003). The only known binding partner of the SART3 HAT domain is hPrp3 but its role in U6 or U4 snRNP targeting was not tested (Medenbach et al. 2004). SART3 is likely bound in CBs through protein–protein interactions because its localization is insensitive to an RNase treatment (Schaffert et al. 2004). Therefore, we consider it likely that the SART3 HAT domain targets the U6 snRNP to CBs through interactions with an unknown binding partner X (see Fig. 3). U4 snRNA localization to CBs is independent of U6 (Stanek and Neugebauer 2004; M. Klingauf, D. S., and K. N., unpublished data), suggesting that the Sm ring alone may be sufficient.

Histone mRNA processing and the U7 snRNP

Although CBs move within nuclei, in situ hybridization has revealed that CBs spend significant time in the vicinity of particular gene loci. Besides snRNA and snoRNA genes, CBs in mammalian tissue culture cells are also associated with histone loci (Frey and Matera 1995; Gao et al. 1997; Jacobs et al. 1999; Schul et al. 1999a; Smith et al. 1995). In amphibian oocyte nuclei, CBs are found attached to histone loci in lampbrush chromosome spreads (Callan et al. 1991). The localization of CBs to histone loci is interesting because CBs also contain the U7 snRNA and the U7snRNP protein LSm10 (Frey and Matera 1995; Pillai et al. 2001; Wu and Gall 1993). The U7 snRNP is a key component of the histone mRNA 3′-end formation machinery and contains a mixture of Sm proteins with two specific LSm proteins (LSm10 and LSm11) in its ring (Pillai et al. 2003). Thus, the U7 snRNP is likely targeted to CBs through interactions with coilin's Sm binding region (Wu et al. 1996; Xu et al. 2005). In addition, LSm10 and LSm11 interact with the SMN complex for assembly (Azzouz et al. 2005; Pillai et al. 2003), suggesting that the U7 snRNP Sm/LSm ring is either assembled in the cytoplasm, analogous to the spliceosomal snRNPs, or in nuclei, perhaps even in CBs. The demonstration that overexpression of the U7 snRNA (but not histone mRNA) leads to the production of new CBs in *Xenopus* oocyte nuclei is consistent with the possibility that the U7 snRNP assembles in CBs (Tuma and Roth 1999). The U7 snRNP and stem loop binding protein (SLBP) recognize elements in the histone mRNA 3′-end and target it for cleavage. The fact that SLBP is also detectable in CBs (Abbott et al. 1999) and that histone 3′-end formation is cotranscriptional (Adamson and Price 2003), suggests that CBs may localize to histone loci via binding to nascent histone transcripts.

Recently, a complex involved in 3′-end processing of histone pre-mRNA was identified and individual components were revealed. The complex includes all CPSF subunits and two CstF proteins with CPSF-73 the likely cleavage factor (Dominski et al. 2005; Kolev and Steitz 2005). Some of the proteins from this complex were localized in cleavage bodies, which are found in close proximity to CBs (Schul et al. 1996). Moreover, Cajal and cleavage body appearance is cell cycle-dependent and cleavage bodies are found specifically adjacent to the Cajal bodies associated with histone genes (Carmo-Fonseca et al. 1993; Fernandez et al. 2002; Schul et al. 1999b). Taken together, these observations support the proposal that CBs and/or adjacent cleavage bodies are sites of histone 3′-end processing.

Life without coilin

Since its discovery in 1990, coilin's function remains mysterious. Coilin, identified in vertebrates (*Xenopus*, zebrafish, and mammals) but not in flies, worms, or fungi, lacks any obvious homology with other proteins (Tucker et al. 2000). Early hints came from mutational analysis, which showed that the N terminus of coilin is necessary for CB formation while the C terminus is necessary for accumulation of spliceosomal snRNPs in CBs (Bohmann et al. 1995). More detailed studies revealed that the N terminus contains a coilin self-association domain and the C terminus contains domains interacting with SMN and spliceosomal snRNPs (Hebert and Matera 2000; Hebert et al. 2001; Xu et al. 2005). However, coilin self-interaction itself cannot explain CB formation because coilin–coilin interaction was detected both in CBs and in the nucleoplasm (Stanek and Neugebauer 2004). In parallel with the coilin mutation strategy, coilin depletion approaches were taken. Bauer and Gall 1997 showed that CB-like structures form in *Xenopus* egg extracts depleted of coilin. However, spliceosomal snRNPs were not localized in these structures, showing that coilin is necessary for snRNP targeting to CBs. Mice lacking functional coilin exhibit reduced viability (Tucker et al. 2001). Cells derived from these mice lack classical CBs; instead, they form several so-called residual CBs, which accumulate different fractions of factors normally present in CBs but fail to concentrate spliceosomal snRNPs (Jady et al. 2003; Stanek and Neugebauer 2004; Tucker et al. 2001). It is interesting to note that the SART3•U4/U6 snRNP intermediate normally concentrated in CBs (see above) is robustly detected by FRET in the nucleoplasm of cells lacking coilin, indicating that these cells compensate for the lack of CBs by performing snRNP assembly in the nucleoplasm (Stanek and Neugebauer 2004). Although a *Drosophila* ortholog of coilin was not identified by sequence similarity, fly nuclei contain nuclear structures resembling mammalian CBs in which spliceosomal snRNPs, scaRNAs, SMN, and fibrillarin are concentrated (Liu et al. 2006; Richard et al. 2003). It is interesting to note that the *Drosophila* U7 snRNP accumulates in a separate nuclear body, which associates with histone genes (Liu et al. 2006). If *Drosophila* truly lacks a functional coilin homologue, these observations

together with the results of coilin depletion experiments in frog and mouse, suggest that coilin brings together distinct sets of factors, which tend to assemble into nuclear bodies even in the absence of coilin. Coilin is highly expressed in testis and CBs were observed as early as in the parental pronuclei of 1-cell embryos (Ferreira and Carmo-Fonseca 1995; Tucker et al. 2001). The fact that coilin knockout mice produce litters significantly smaller that wild type (Tucker et al. 2001) suggests a role for coilin and CBs in either fertility or early embryonic development.

Conclusions and perspective

Here, we have summarized recent evidence that spliceosomal snRNP assembly occurs in CBs, focusing on the U4/U6 snRNP and the U4/U6•U5 snRNP as examples (Fig. 3). Similar principles apply to U2 snRNP assembly and possibly to the assembly of additional RNPs such as the U7 snRNP. CBs appear to be a meeting place for precursors—either newly synthesized or disassembled after splicing—that may assemble more efficiently due to higher local concentrations of components. The CB may provide a "catalytic surface" within nuclei upon which biochemical events, such as U4/U6 snRNP assembly, more favorably occur. Sequestering of snRNP assembly intermediates in CBs may also prevent their interference with spliceosome assembly in the nucleoplasm.

Beyond the CB, these insights also suggest a similar logic for the concentration of different sets of factors in other nuclear bodies. Indeed, distinct nonmembrane-bound bodies, such as P granules, stress granules, and recently P bodies, were discovered in the cytoplasm and raise similar questions as to localization and function. Further application of advanced light microscopy approaches, such as FRET and fluorescence correlation spectroscopy, will help reveal how nonmembrane-bound structures are formed and maintained. Thus, the CB also provides a meeting place for scientists seeking to understand the dynamics and functions of both nuclear and cytoplasmic bodies.

Acknowledgements Work in our laboratories is supported by the Max Planck Society, a grant (to KMN) from the Deutsche Forschung Gemeinschaft (NE909/1-1), and grants (to DS) from Granting Agency of the Czech Republic (301/05/0601) and the Czech Ministry of Education (1K05009, MSM0021620806, AV0Z50110509 and LC535). We thank Christine Panagiotidis for help with graphics and Mirko Klingauf, Magda Strzelecka, and Arnold Kiss for helpful discussions and comments on the manuscript.

References

Abbott J, Marzluff WF, Gall JG (1999) The stem-loop binding protein (SLBP1) is present in coiled bodies of the *Xenopus* germinal vesicle. Mol Biol Cell 10:487–499

Achsel T, Brahms H, Kastner B, Bachi A, Wilm M, Luhrmann R (1999) A doughnut-shaped heteromer of human Sm-like proteins binds to the 3′- end of U6 snRNA, thereby facilitating U4/U6 duplex formation in vitro. EMBO J 18:5789–5802

Adamson TE, Price DH (2003) Cotranscriptional processing of Drosophila histone mRNAs. Mol Cell Biol 23:4046–4055

Andrade LE, Chan EK, Raska I, Peebles CL, Roos G, Tan EM (1991) Human autoantibody to a novel protein of the nuclear coiled body: immunological characterization and cDNA cloning of p80-coilin. J Exp Med 173:1407–1419

Andrade LE, Tan EM, Chan EK (1993) Immunocytochemical analysis of the coiled body in the cell cycle and during cell proliferation. Proc Natl Acad Sci USA 90:1947–1951

Azzouz TN, Pillai RS, Dapp C, Chari A, Meister G, Kambach C, Fischer U, Schumperli D (2005) Toward an assembly line for U7 snRNPs: interactions of U7-specific Lsm proteins with PRMT5 and SMN complexes. J Biol Chem 280:34435–34440

Baillat D, Hakimi MA, Naar AM, Shilatifard A, Cooch N, Shiekhattar R (2005) Integrator, a multiprotein mediator of small nuclear RNA processing, associates with the C-terminal repeat of RNA polymerase II. Cell 123:265–276

Bauer DW, Gall JG (1997) Coiled bodies without coilin. Mol Biol Cell 8:73–82

Behrens SE, Luhrmann R (1991) Immunoaffinity purification of a [U4/U6.U5] tri-snRNP from human cells. Genes Dev 5: 1439–1452

Bell M, Schreiner S, Damianov A, Reddy R, Bindereif A (2002) p110, a novel human U6 snRNP protein and U4/U6 snRNP recycling factor. EMBO J 21:2724–2735

Bohmann K, Ferreira JA, Lamond AI (1995) Mutational analysis of p80 coilin indicates a functional interaction between coiled bodies and the nucleolus. J Cell Biol 131:817–831

Boudonck K, Dolan L, Shaw PJ (1998) Coiled body numbers in the Arabidopsis root epidermis are regulated by cell type, developmental stage and cell cycle parameters. J Cell Sci 111: 3687–3694

Boudonck K, Dolan L, Shaw PJ (1999) The movement of coiled bodies visualized in living plant cells by the green fluorescent protein. Mol Biol Cell 10:2297–2307

Boulon S, Verheggen C, Jady BE, Girard C, Pescia C, Paul C, Ospina JK, Kiss T, Matera AG, Bordonne R, Bertrand E (2004) PHAX and CRM1 are required sequentially to transport U3 snoRNA to nucleoli. Mol Cell 16:777–787

Brosi R, Groning K, Behrens SE, Luhrmann R, Kramer A (1993) Interaction of mammalian splicing factor SF3a with U2 snRNP and relation of its 60-kD subunit to yeast PRP9. Science 262:102–105

Cajal RY (1903) Un sencillo metodo de coloracion seletiva del reticulo protoplasmatico y sus efectos en los diversos organos nerviosos de vertebrados e invertebrados. Trab Lab Investig Biol Univ Madr 2:129–221

Callan HG, Gall JG, Murphy C (1991) Histone genes are located at the sphere loci of *Xenopus* lampbrush chromosomes. Chromosoma 101:245–251

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

Carmo-Fonseca M (2002) New clues to the function of the Cajal body. EMBO Rep 3:726–727

Carmo-Fonseca M, Pepperkok R, Carvalho MT, Lamond AI (1992) Transcription-dependent colocalization of the U1, U2, U4/U6, and U5 snRNPs in coiled bodies. J Cell Biol 117:1–14

Carmo-Fonseca M, Ferreira J, Lamond AI (1993) Assembly of snRNP-containing coiled bodies is regulated in interphase and mitosis—evidence that the coiled body is a kinetic nuclear structure. J Cell Biol 120:841–852

Carmo-Fonseca M, Platani M, Swedlow JR (2002) Macromolecular mobility inside the cell nucleus. Trends Cell Biol 12:491–495

Carvalho T, Almeida F, Calapez A, Lafarga M, Berciano MT, Carmo-Fonseca M (1999) The spinal muscular atrophy disease gene product, SMN: a link between snRNP biogenesis and the Cajal (coiled) body. J Cell Biol 147:715–728

Chan SP, Kao DI, Tsai WY, Cheng SC (2003) The Prp19p-associated complex in spliceosome activation. Science 302: 279–282

Cioce M, Lamond AI (2005) Cajal bodies: a long history of discovery. Annu Rev Cell Dev Biol 21:105-131

Damianov A, Schreiner S, Bindereif A (2004) Recycling of the U12-type spliceosome requires p110, a component of the U6atac snRNP. Mol Cell Biol 24:1700–1708

Darzacq X, Jady BE, Verheggen C, Kiss AM, Bertrand E, Kiss T (2002) Cajal body-specific small nuclear RNAs: a novel class of 2′-*O*- methylation and pseudouridylation guide RNAs. EMBO J 21:2746–2756

Deryusheva S, Gall JG (2004) Dynamics of coilin in Cajal bodies of the *Xenopus* germinal vesicle. Proc Natl Acad Sci USA 101:4810–4814

Dominski Z, Yang XC, Marzluff WF (2005) The polyadenylation factor CPSF-73 is involved in histone-pre-mRNA processing. Cell 123:37–48

Dundr M, Hebert MD, Karpova TS, Stanek D, Xu H, Shpargel KB, Meier UT, Neugebauer KM, Matera AG, Misteli T (2004) In vivo kinetics of Cajal body components. J Cell Biol 164: 831–842

Feeney RJ, Zieve GW (1990) Nuclear exchange of the U1 and U2 snRNP-specific proteins. J Cell Biol 110:871–881

Fernandez R, Pena E, Navascues J, Casafont I, Lafarga M, Berciano MT (2002) cAMP-dependent reorganization of the Cajal bodies and splicing machinery in cultured Schwann cells. Glia 40:378–388

Ferreira J, Carmo-Fonseca M (1995) The biogenesis of the coiled body during early mouse development. Development 121: 601–612

Frey MR, Matera AG (1995) Coiled bodies contain U7 small nuclear RNA and associate with specific DNA sequences in interphase human cells. Proc Natl Acad Sci USA 92: 5915–5919

Frey MR, Matera AG (2001) RNA-mediated interaction of Cajal bodies and U2 snRNA genes. J Cell Biol 154:499–509

Frey MR, Bailey AD, Weiner AM, Matera AG (1999) Association of snRNA genes with coiled bodies is mediated by nascent snRNA transcripts. Curr Biol 9:126–135

Friesen WJ, Massenet S, Paushkin S, Wyce A, Dreyfuss G (2001) SMN, the product of the spinal muscular atrophy gene, binds preferentially to dimethylarginine-containing protein targets. Mol Cell 7:1111–1117

Gall JG (2000) Cajal bodies: the first 100 years. Annu Rev Cell Dev Biol 16:273–300

Gangwani L, Flavell RA, Davis RJ (2005) ZPR1 is essential for survival and is required for localization of the survival motor neurons (SMN) protein to Cajal bodies. Mol Cell Biol 25: 2744–2756

Ganot P, Jady BE, Bortolin ML, Darzacq X, Kiss T (1999) Nucleolar factors direct the 2′-*O*-ribose methylation and pseudouridylation of U6 spliceosomal RNA. Mol Cell Biol 19: 6906–6917

Gao L, Frey MR, Matera AG (1997) Human genes encoding U3 snRNA associate with coiled bodies in interphase cells and are clustered on chromosome 17p11.2 in a complex inverted repeat structure. Nucleic Acids Res 25:4740–4747

Gerbi SA, Borovjagin AV, Lange TS (2003) The nucleolus: a site of ribonucleoprotein maturation. Curr Opin Cell Biol 15:318–325

Grimmler M, Otter S, Peter C, Muller F, Chari A, Fischer U (2005) Unrip, a factor implicated in cap-independent translation, associates with the cytosolic SMN complex and influences its intracellular localization. Hum Mol Genet 14:3099–3111

Handwerger KE, Murphy C, Gall JG (2003) Steady-state dynamics of Cajal body components in the *Xenopus* germinal vesicle. J Cell Biol 160:495–504

Hebert MD, Matera AG (2000) Self-association of coilin reveals a common theme in nuclear body localization. Mol Biol Cell 11:4159–4171

Hebert MD, Szymczyk PW, Shpargel KB, Matera AG (2001) Coilin forms the bridge between Cajal bodies and SMN, the spinal muscular atrophy protein. Genes Dev 15:2720–2729

Hebert MD, Shpargel KB, Ospina JK, Tucker KE, Matera AG (2002) Coilin methylation regulates nuclear body formation. Dev Cell 3:329–337

Hoet RM, Raats JM, de Wildt R, Dumortier H, Muller S, van den Hoogen F, van Venrooij WJ (1998) Human monoclonal autoantibody fragments from combinatorial antibody libraries directed to the U1snRNP associated U1C protein; epitope mapping, immunolocalization and V-gene usage. Mol Immunol 35:1045–1055

Horowitz DS, Kobayashi R, Krainer AR (1997) A new cyclophilin and the human homologues of yeast Prp3 and Prp4 form a complex associated with U4/U6 snRNPs. RNA 3:1374–1387

Jacobs EY, Frey MR, Wu W, Ingledue TC, Gebuhr TC, Gao L, Marzluff WF, Matera AG (1999) Coiled bodies preferentially associate with U4, U11, and U12 small nuclear RNA genes in interphase HeLa cells but not with U6 and U7 genes. Mol Biol Cell 10:1653–1663

Jacobs EY, Ogiwara I, Weiner AM (2004) Role of the C-terminal domain of RNA polymerase II in U2 snRNA transcription and 3′ processing. Mol Cell Biol 24:846–855

Jady 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

Jady BE, Richard P, Bertrand E, Kiss T (2005) Cell cycle-dependent recruitment of telomerase RNA and Cajal bodies to human telomeres. Mol Biol Cell 17:944–954

Jantsch MF, Gall JG (1992) Assembly and localization of the U1-specific snRNP C protein in the amphibian oocyte. J Cell Biol 119:1037–1046

Jurica MS, Moore MJ (2003) Pre-mRNA splicing: awash in a sea of proteins. Mol Cell 12:5–14

Jurica MS, Licklider LJ, Gygi SR, Grigorieff N, Moore MJ (2002) Purification and characterization of native spliceosomes suitable for three-dimensional structural analysis. RNA 8:426–439

Kambach C, Mattaj IW (1992) Intracellular distribution of the U1A protein depends on active transport and nuclear binding to U1 snRNA. J Cell Biol 118:11–21

Kambach C, Mattaj IW (1994) Nuclear transport of the U2 snRNP-specific U2B″ protein is mediated by both direct and indirect signalling mechanisms. J Cell Sci 107(Pt 7):1807–1816

Kiss T (2004) Biogenesis of small nuclear RNPs. J Cell Sci 117:5949–5951

Kiss AM, Jady BE, Darzacq X, Verheggen C, Bertrand E, Kiss T (2002) A Cajal body-specific pseudouridylation guide RNA is composed of two box H/ACA snoRNA-like domains. Nucleic Acids Res 30:4643–4649

Kolev NG, Steitz JA (2005) Symplekin and multiple other polyadenylation factors participate in 3′-end maturation of histone mRNAs. Genes Dev 19:2583–2592

Kramer A, Gruter P, Groning K, Kastner B (1999) Combined biochemical and electron microscopic analyses reveal the architecture of the mammalian U2 snRNP. J Cell Biol 145: 1355–1368

Laggerbauer B, Liu S, Makarov E, Vornlocher HP, Makarova O, Ingelfinger D, Achsel T, Luhrmann R (2005) The human U5 snRNP 52K protein (CD2BP2) interacts with U5-102K (hPrp6), a U4/U6.U5 tri-snRNP bridging protein, but dissociates upon tri-snRNP formation. RNA 11:598–608

Lange TS, Gerbi SA (2000) Transient nucleolar localization Of U6 small nuclear RNA in *Xenopus laevis* oocytes. Mol Biol Cell 11:2419–2428

Lewis JD, Tollervey D (2000) Like attracts like: getting RNA processing together in the nucleus. Science 288:1385–1389

Liu JL, Murphy C, Buszczak M, Clatterbuck S, Goodman R, Gall JG (2006) The Drosophila Cajal body. J Cell Biol Vol 172 (in press)

Makarov EM, Makarova OV, Urlaub H, Gentzel M, Will CL, Wilm M, Luhrmann R (2002) Small nuclear ribonucleoprotein remodeling during catalytic activation of the spliceosome. Science 298:2205–2208

Makarova OV, Makarov EM, Liu S, Vornlocher HP, Luhrmann R (2002) Protein 61K, encoded by a gene (PRPF31) linked to autosomal dominant retinitis pigmentosa, is required for U4/U6 center dotU5 tri-snRNP formation and pre-mRNA splicing. EMBO J 21:1148–1157

Makarova OV, Makarov EM, Urlaub H, Will CL, Gentzel M, Wilm M, Luhrmann R (2004) A subset of human 35S U5 proteins, including Prp19, function prior to catalytic step 1 of splicing. EMBO J 23:2381–2391

Massenet S, Pellizzoni L, Paushkin S, Mattaj IW, Dreyfuss G (2002) The SMN complex is associated with snRNPs throughout their cytoplasmic assembly pathway. Mol Cell Biol 22:6533–6541

Matera AG (1998) Of coiled bodies, gems, and salmon. J Cell Biochem 70:181–192

Matera AG (1999) Nuclear bodies: multifaceted subdomains of the interchromatin space. Trends Cell Biol 9:302–309

Matera AG, Ward DC (1993) Nucleoplasmic organization of small nuclear ribonucleoproteins in cultured human cells. J Cell Biol 121:715–727

Medenbach, Schreiner S, Liu S, Luhrmann R, Bindereif A (2004) Human U4/U6 snRNP recycling factor p110: mutational analysis reveals function of TPR domain in recycling. Mol Cell Biol 24:7392–7401

Medlin JE, Uguen P, Taylor A, Bentley DL, Murphy S (2003) The C-terminal domain of pol II and a DRB-sensitive kinase are required for 3′ processing of U2 snRNA. EMBO J 22:925–934

Meister G, Eggert C, Fischer U (2002) SMN-mediated assembly of RNPs: a complex story. Trends Cell Biol 12:472–478

Misteli T (2001) The concept of self-organization in cellular architecture. J Cell Biol 155:181–185

Molenaar C, Abdulle A, Gena A, Tanke HJ, Dirks RW (2004) Poly (A)+ RNAs roam the cell nucleus and pass through speckle domains in transcriptionally active and inactive cells. J Cell Biol 165:191–202

Monneron A, Bernhard W (1969) Fine structural organization of the interphase nucleus in some mammalian cells. J Ultrastruct Res 27:266–288

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, Luhrmann R, Matera AG (2004) Coupled in vitro import of U snRNPs and SMN, the spinal muscular atrophy protein. Mol Cell 16:223–234

Nelissen RL, Will CL, van Venrooij WJ, Luhrmann R (1994) The association of the U1-specific 70K and C proteins with U1 snRNPs is mediated in part by common U snRNP proteins. EMBO J 13:4113–4125

Nesic D, Kramer A (2001) Domains in human splicing factors SF3a60 and SF3a66 required for binding to SF3a120, assembly of the 17S U2 snRNP, and prespliceosome formation. Mol Cell Biol 21:6406–6417

Nesic D, Tanackovic G, Kramer A (2004) A role for Cajal bodies in the final steps of U2 snRNP biogenesis. J Cell Sci 117: 4423–4433

Neugebauer KM (2002) On the importance of being co-transcriptional. J Cell Sci 115:3865–3871

Nottrott S, Urlaub H, Luhrmann R (2002) Hierarchical, clustered protein interactions with U4/U6 snRNA: a biochemical role for U4/U6 proteins. EMBO J 21:5527–5538

Ogg SC, Lamond AI (2002) Cajal bodies and coilin-moving towards function. J Cell Biol 159:17–21

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

Paushkin S, Gubitz AK, Massenet S, Dreyfuss G (2002) The SMN complex, an assemblyosome of ribonucleoproteins. Curr Opin Cell Biol 14:305–312

Pellizzoni L, Kataoka N, Charroux B, Dreyfuss G (1998) A novel function for SMN, the spinal muscular atrophy disease gene product, in pre-mRNA splicing. Cell 95:615–624

Pillai RS, Will CL, Luhrmann R, Schumperli D, Muller B (2001) Purified U7 snRNPs lack the Sm proteins D1 and D2 but contain Lsm10, a new 14 kDa Sm D1-like protein. EMBO J 20:5470–5479

Pillai RS, Grimmler M, Meister G, Will CL, Luhrmann R, Fischer U, Schumperli D (2003) Unique Sm core structure of U7 snRNPs: assembly by a specialized SMN complex and the role of a new component, Lsm11, in histone RNA processing. Genes Dev 17:2321–2333

Platani M, Goldberg I, Swedlow JR, Lamond AI (2000) In vivo analysis of Cajal body movement, separation, and joining in live human cells. J Cell Biol 151:1561–1574

Platani M, Goldberg I, Lamond AI, Swedlow JR (2002) Cajal body dynamics and association with chromatin are ATP-dependent. Nat Cell Biol 4:502–508

Rader SD, Guthrie C (2002) A conserved Lsm-interaction motif in Prp24 required for efficient U4/U6 di-snRNP formation. RNA 8:1378–1392

Raska I, Andrade LE, Ochs RL, Chan EK, Chang CM, Roos G, Tan EM (1991) Immunological and ultrastructural studies of the nuclear coiled body with autoimmune antibodies. Exp Cell Res 195:27–37

Richard P, Darzacq X, Bertrand E, Jady BE, Verheggen C, Kiss T (2003) A common sequence motif determines the Cajal body-specific localization of box H/ACA scaRNAs. EMBO J 22: 4283–4293

Schaffert N, Hossbach M, Heintzmann R, Achsel T, Luhrmann R (2004) RNAi knockdown of hPrp31 leads to an accumulation of U4/U6 di-snRNPs in Cajal bodies. EMBO J 23:3000–3009

Schneider C, Will CL, Makarova OV, Makarov EM, Luhrmann R (2002) Human U4/U6.U5 and U4atac/U6atac.U5 tri-snRNPs exhibit similar protein compositions. Mol Cell Biol 22: 3219–3229

Schul W, Groenhout B, Koberna K, Takagaki Y, Jenny A, Manders EM, Raska I, van Driel R, de Jong L (1996) The RNA 3′ cleavage factors CstF 64 kDa and CPSF 100 kDa are concentrated in nuclear domains closely associated with coiled bodies and newly synthesized RNA. EMBO J 15:2883–2892

Schul W, van Driel R, de Jong L (1998) Coiled bodies and U2 snRNA genes adjacent to coiled bodies are enriched in factors required for snRNA transcription. Mol Biol Cell 9:1025–1036

Schul W, Adelaar B, van Driel R, de Jong L (1999a) Coiled bodies are predisposed to a spatial association with genes that contain snoRNA sequences in their introns. J Cell Biochem 75: 393–403

Schul W, van Der Kraan I, Matera AG, van Driel R, de Jong L (1999b) Nuclear domains enriched in RNA 3′-processing factors associate with coiled bodies and histone genes in a cell cycle-dependent manner. Mol Biol Cell 10:3815–3824

Schumperli D, Pillai RS (2004) The special Sm core structure of the U7 snRNP: far-reaching significance of a small nuclear ribonucleoprotein. Cell Mol Life Sci 61:2560–2570

Segref A, Mattaj IW, Ohno M (2001) The evolutionarily conserved region of the U snRNA export mediator PHAX is a novel RNA-binding domain that is essential for U snRNA export. RNA 7:351–360

Shav-Tal Y, Singer RH, Darzacq X (2004) Imaging gene expression in single living cells. Nat Rev Mol Cell Biol 5:855–861

Shpargel KB, Matera AG (2005) Gemin proteins are required for efficient assembly of Sm-class ribonucleoproteins. Proc Natl Acad Sci USA 102:17372–17377

Singh R, Reddy R (1989) Gamma-monomethyl phosphate: a cap structure in spliceosomal U6 small nuclear RNA. Proc Natl Acad Sci USA 86:8280–8283

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

Sleeman JE, Ajuh P, Lamond AI (2001) snRNP protein expression enhances the formation of Cajal bodies containing p80-coilin and SMN. J Cell Sci 114:4407–4419

Sleeman JE, Trinkle-Mulcahy L, Prescott AR, Ogg SC, Lamond AI (2003) Cajal body proteins SMN and /coilin show differential dynamic behaviour in vivo. J Cell Sci 116:2039–2050

Smith KP, Lawrence JB (2000) Interactions of U2 gene loci and their nuclear transcripts with Cajal (coiled) bodies: evidence for PreU2 within Cajal bodies. Mol Biol Cell 11:2987–2998

Smith KP, Carter KC, Johnson CV, Lawrence JB (1995) U2 and U1 snRNA gene loci associate with coiled bodies. J Cell Biochem 59:473–485

Staley JP, Guthrie C (1998) Mechanical devices of the spliceosome: motors, clocks, springs, and things. Cell 92:315–326

Stanek D, Neugebauer KM (2004) Detection of snRNP assembly intermediates in Cajal bodies by fluorescence resonance energy transfer. J Cell Biol 166:1015–1025

Stanek D, Rader SD, Klingauf M, Neugebauer KM (2003) Targeting of U4/U6 small nuclear RNP assembly factor SART3/p110 to Cajal bodies. J Cell Biol 160:505–516

Teigelkamp S, Achsel T, Mundt C, Gothel SF, Cronshagen U, Lane WS, Marahiel M, Luhrmann R (1998) The 20 kD protein of human [U4/U6.U5] tri-snRNPs is a novel cyclophilin that forms a complex with the U4/U6-specific 60 kD and 90 kD proteins. RNA 4:127–141

Terns MP, Terns RM (2001) Macromolecular complexes: SMN–the master assembler. Curr Biol 11:R862–R864

Tucker KE, Matera AG (2005) The Cajal body: a nuclear gathering place. In: Hemmerich P, Diekmann S (eds) Vision of the cell nucleus. American Scientific, California, USA

Tucker KE, Massello LK, Gao L, Barber TJ, Hebert MD, Chan EK, Matera AG (2000) Structure and characterization of the murine p80 coilin gene, coil. J Struct Biol 129:269–277

Tucker KE, Berciano MT, Jacobs EY, LePage DF, Shpargel KB, Rossire JJ, Chan EK, Lafarga M, Conlon RA, Matera AG (2001) Residual Cajal bodies in coilin knockout mice fail to recruit Sm snRNPs and SMN, the spinal muscular atrophy gene product. J Cell Biol 154:293–307

Tuma RS, Roth MB (1999) Induction of coiled body-like structures in *Xenopus* oocytes by U7 snRNA. Chromosoma 108:337–344

Tuma RS, Stolk JA, Roth MB (1993) Identification and characterization of a sphere organelle protein. J Cell Biol 122:767–773

Tycowski KT, You ZH, Graham PJ, Steitz JA (1998) Modification of U6 spliceosomal RNA is guided by other small RNAs. Mol Cell 2:629–638

Uguen P, Murphy S (2003) The 3′ ends of human pre-snRNAs are produced by RNA polymerase II CTD-dependent RNA processing. EMBO J 22:4544–4554

Watkins NJ, Segault V, Charpentier B, Nottrott S, Fabrizio P, Bachi A, Wilm M, Rosbash M, Branlant C, Luhrmann R (2000) A common core RNP structure shared between the small nucleoar box C/D RNPs and the spliceosomal U4 snRNP. Cell 103: 457–466

Watkins NJ, Lemm I, Ingelfinger D, Schneider C, Hossbach M, Urlaub H, Luhrmann R (2004) Assembly and maturation of the U3 snoRNP in the nucleoplasm in a large dynamic multiprotein complex. Mol Cell 16:789–798

Will CL, Luhrmann R (2005) Splicing of a rare class of introns by the U12-dependent spliceosome. Biol Chem 386:713–724

Will CL, Urlaub H, Achsel T, Gentzel M, Wilm M, Luhrmann R (2002) Characterization of novel SF3b and 17S U2 snRNP proteins, including a human Prp5p homologue and an SF3b DEAD-box protein. EMBO J 21:4978–4988

Will CL, Schneider C, Hossbach M, Urlaub H, Rauhut R, Elbashir S, Tuschl T, Luhrmann R (2004) The human 18S U11/U12 snRNP contains a set of novel proteins not found in the U2-dependent spliceosome. RNA 10:929–941

Wu CH, Gall JG (1993) U7 small nuclear RNA in C snurposomes of the *Xenopus* germinal vesicle. Proc Natl Acad Sci USA 90:6257–6259

Wu CH, Murphy C, Gall JG (1996) The Sm binding site targets U7 snRNA to coiled bodies (spheres) of amphibian oocytes. RNA 2:811–823

Xu H, Pillai RS, Azzouz TN, Shpargel KB, Kambach C, Hebert MD, Schumperli D, Matera AG (2005) The C-terminal domain of coilin interacts with Sm proteins and U snRNPs. Chromosoma 114:155–166

Yan D, Perriman R, Igel H, Howe KJ, Neville M, Ares M, Jr. (1998) CUS2, a yeast homolog of human Tat-SF1, rescues function of misfolded U2 through an unusual RNA recognition motif. Mol Cell Biol 18:5000–5009

Yu YT, Shu MD, Steitz JA (1998) Modifications of U2 snRNA are required for snRNP assembly and pre-mRNA splicing. EMBO J 17:5783–5795

Zaric B, Chami M, Remigy H, Engel A, Ballmer-Hofer K, Winkler FK, Kambach C (2005) Reconstitution of two recombinant LSm protein complexes reveals aspects of their architecture, assembly, and function. J Biol Chem 280:16066–16075

Zhao X, Yu YT (2004) Pseudouridines in and near the branch site recognition region of U2 snRNA are required for snRNP biogenesis and pre-mRNA splicing in *Xenopus* oocytes. RNA 10:681–690