The recruitment of the U5 snRNP to nascent transcripts requires internal loop 1 of U5 snRNA.

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Running title: Recruiting U5 snRNP to nascent transcripts

Abbreviations:
CB: Cajal body
IGC: interchromatin granule cluster
IL: Internal Loop
LBC: lampbrush chromosomes
RNAPII: RNA polymerase II
SL: Stem Loop
snRNP: small nuclear ribonucleoprotein particle
snRNA: small nuclear RNA

Keywords: Cajal bodies, Lampbrush chromosomes, RNA splicing, spliceosome, snRNAs

Abstract

In this study, we take advantage of the high spatial resolution offered by the nucleus and lampbrush chromosomes of the amphibian oocyte to investigate the mechanism that regulates the intranuclear trafficking of the U5 snRNP and its recruitment to nascent transcripts. We monitor the fate of newly assembled fluorescent U5 snRNP in *Xenopus* oocytes depleted of U4 and/or U6 snRNAs and demonstrate that the U4/U6.U5 tri-snRNP is not required for the association of U5 snRNP with Cajal bodies, splicing speckles and nascent transcripts. In addition, using a mutational analysis, we show that a non-functional U5 snRNP can associate with nascent transcripts, and we further characterize internal loop structure 1 (IL1) of U5 snRNA as a critical element for licensing U5 snRNP to target both nascent transcripts and splicing speckles. Collectively, our data support the model where the recruitment of snRNPs onto pre-mRNAs is independent of spliceosome assembly and suggest that U5 snRNP may promote the association of the U4/U6.U5 tri-snRNP with nascent transcripts.

Introduction

One of the most prominent features of the lampbrush chromosomes (LBCs) is their very high level of RNA polymerase II (RNAPII) activity (reviewed in Callan, 1986; Morgan, 2002; see Macgregor *ibid*). This property is structurally showcased by numerous transcription units in the form of loops that are projected laterally, away from the relatively condensed chromosomal axes. While individual loops are easily distinguished using a light microscope, it is important to note that their chromatin axes are only revealed by labeling with antibodies against DNA (see Morgan, *ibid*) or proteins associated with DNA, such as histones (Austin *et al.*, 2009) and active RNAPII (Gall *et al.*, 1999, see Gall *ibid*). In essence, the lateral loops correspond to nascent RNAPII transcripts that are associated with numerous RNA processing and export factors. Together, they create a ribonucleoprotein matrix, which is dense enough to be seen by phase contrast or DIC, surrounding a highly decondensed chromatin axis. LBC loops, therefore, represent a unique cellular system to investigate the mechanisms controlling the major steps in RNA transcription and processing, particularly the recruitment of the various machineries involved in these processes. Indeed, it has become well accepted that pre-mRNA transcription and maturation, including splicing, are two interrelated processes in all eukaryotes (Bentley, 2005; de Almeida and Carmo-Fonseca, 2008; Martins *et al.*, 2011).

A critical step in pre-mRNA maturation is the removal of introns and ligation of exons by the spliceosome, a multi-subunit and dynamic enzyme (Zhou *et al.*, 2002;

Jurica and Moore, 2003; Nilsen, 2003; Patel and Steitz, 2003). At the heart of the spliceosome are its catalytic components, the five major small nuclear ribonucleoproteins U1, U2, U4, U5 and U6 snRNPs. Each spliceosomal snRNP consists of a small nuclear RNA (snRNA) associated with a specific set of proteins. The biogenesis of most snRNPs follows a complex maturation pathway that involves both a cytoplasmic and nuclear phase (Patel and Bellini, 2008). Within the nucleus, snRNPs are often detected in association with several discrete domains such as Cajal bodies (CBs), nucleoli, and interchromatin granule clusters (IGCs). While the functional implications of these associations are not immediately clear, the current view is that these domains are involved in the maturation and/or storage of snRNPs and other processing factors prior to their recruitment to nascent transcripts. Previously, we demonstrated that the recruitment of the splicing snRNP to nascent transcripts is independent of the spliceosomal assembly (Patel *et al.*, 2007; Paschedag *et al.*, in revision). Additionally, we showed that discrete elements of the U1 and U2 snRNPs control their intranuclear trafficking and recruitment to nascent transcripts (Patel *et al.*, 2007; Paschedag *et al.*, in revision). Here, we extend our analysis to the U5 snRNP, which integrates the spliceosome as part of the U4/U6-U5 tri-snRNP complex. Interestingly, we find that the U5 snRNP still targets nascent transcripts in absence of U4 and/or U6 snRNP. In addition, we define the small internal loop 1 (IL1) of U5 snRNA as a critical element for association with both IGCs and nascent transcripts.

Materials and Methods:

In vitro transcription and labeling

The cDNA clone coding for the full length *Xenopus laevis* U5 snRNA was generously provided by Dr. Joseph Gall, and was used to generate all templates for *in vitro* transcription of the wild-type and mutant U5 snRNAs. DNA templates were obtained by PCR, and in all cases the T3 promoter was introduced immediately upstream of the sequence to be transcribed. DNA primers (Integrated DNA Technologies) used were as follows (T3 promoter is underlined):

U5 WT: 5'CGGAATTCAATTAACCCTCACTAAAGGG and 3'ATACCTGGTGTGA ACCAGGCTTC

U5Δ5'47: 5'CGAATTAACCCTCACTAAAGGAAAGATTTCCGTGG and 3'ATACCT GGTGTGAACCAGGCTTC

U5Δ5'68: CGAATTAACCCTCACTAAAGGCGACCATGAGTTTCG and 3'ATACCT GGTGTGAACCAGGCTTC

U5Δ3'96: 5'CGGAATTCAATTAACCCTCACTAAAGGG and 3'TTCAAAAAATTG AACGAAACTCATGGTCG

ΔSL1: Step 1: 5'CCTCTGGTTTCTCTTCAAATTCGAATAAATCTTTTTCG AAAGATTTCCG and 3'ATACCTGGTGTGAACCAGGCTTC;

Step 2: 5'AATTAACCCTCACTAAAGGATACTCTGGTTTCTCTTCAAATTCG and 3'-ATACCTGGTGTGAACCAGGCTTC

Δ IL1 Step 1: 5'AATTAACCCTCACTAAAGGATACTTTTCTCTTCAAATTCGAAT and 3'TGAACCAGGCTTCAAAAAATTGAACGAAACTTTCCTCTCCACGG

Step2: 5'AATTAACCCTCACTAAAGGATACTTTTCTCTTCAAATTCGAAT and 3'ATACCTGGTGTGAACCAGGCTTC

ΔIL2: Step 1: 5'CGCCTTTTACTAAAGATGAGAGGAACGACCAT and 3'ATACCT GGTGTGAACCAGGCTTC;

Step 2: 5'AATTAACCCTCACTAAAGGATACTCTGGTTTCTCTTATCTTTCGCC TTTTAC and 3'ATACCTGGTGTGAACCAGGCTTC

Amplified DNA templates were gel purified using 0.45 mm cellulose acetate spin-X filters (Corning Inc.), phenol extracted, and ethanol precipitated before transcription. Fluorescently labeled U5 snRNA and mutants were synthesized using T3 polymerase (Stratagene) in the presence of 25 μM fluorescein-12-UTP (Roche), 625 μM ATP and CTP, 312.5 μM UTP, 250 μM GTP, and 1.25 mM m7G(5')ppp(5')G cap analogue (New England Biolabs, Inc.). Reactions were carried out for 3 hours at 37°C, treated with RQ1 RNase-free DNase (Fisher Scientific) for 10 minutes at 37°C, and newly made transcripts were purified on NucAway Spin columns (Ambion).

Oocytes and microinjections

Fragments of ovary were surgically removed from female adult frogs that were anesthetized in 0.15% tricaine methane sulfonate (MS222; Sigma-Aldrich). Oocytes were defolliculated for 2 h at room temperature in saline buffer OR2 (Wallace *et al.*, 1973) containing 0.2% collagenase (type II; Sigma-Aldrich). Stage IV-V oocytes were subsequently isolated and maintained at 18˚C in OR2. All injections were performed directly into the cytoplasm of oocytes with ~10-20 fmol of RNA, with

volumes no greater than 30 nL. For the U4 and U6 depletion experiments, 50ng of U4d and U6f oligonucleotides (Integrated DNA Technologies) were injected per oocyte 4 hours prior to the injection of the fluorescent U5 WT snRNA. The U4d sequence, TATTGGGAAAAGTTT, is complementary to nucleotides 66-80 of U4 snRNA. The U6f sequence, TCGTTCCAATTTTAG, is complementary to nucleotides 25-39 of U6 snRNA. Glass needles were prepared using a horizontal pipette puller (P-97; Sutter Instruments Co.). Injections were performed under a dissecting microscope (Leica), using the nanojetII micro-injector from Drummond.

Nuclear Spreads and Immunofluorescence

Nuclear spreads were prepared as described in (Patel *et al.*, 2007). Preparations were fixed in PBS containing 2% paraformaldehyde and 1mM $MgCl_2$, for 1 h at room temperature, and blocked in PBS with 0.5% BSA (Sigma-Aldrich) + 0.5% gelatin (from cold water fish), for 10 min. The fluorescent signal emitted by fluorescein-labeled RNAs was amplified as follows: Spread preparations were incubated with primary antibody Alexa Fluor 488 anti-fluorescein (Invitrogen) diluted at 2.5µg/ml, washed with PBS, then incubated with secondary antibody Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen) at 2.5 µg/ml. Spread were washed again with PBS and mounted in 50% glycerol in PBS + 1mg/ml phenylene-diamine and 10 pg/ml DAPI. Microscopy was performed using a fluorescent microscope (DMR; Leica) and a Fluotar 100x NA 1.30 oil objective (Leica). Images were captured with a Retiga EXI monochrome CCD camera (QImaging) and In Vivo software (version 3.2.0, Media

Cybernetics), processed with Photoshop CS version 8.0 (Adobe), and assembled with InDesign CS version 3.0 (Adobe).

Northern Blotting

Two sets of 10 oocytes were injected with the U4d and U6f oligos, and 10 uninjected oocytes were used as a control. After 4 hours of incubation at 18°C, the nuclei were collected and homogenized in 10 mM Tri-HCl, pH 8.0, 1mM EDTA, and 0.2% SDS. Total RNAs were phenol extracted, and ethanol precipitated. Northern Blots were performed as described in (Patel *et al.*, 2007) with following modifications to the protocol: the membranes were cross-linked at 120mJ/cm^2, and antisense U4, U5, and U6 snRNAs were used as probes.

Results

Newly assembled U5 snRNPs associates with CBs, IGCs and LBC loops. In a recent work using *Xenopus* oocytes, we showed that injection of fluorescent U2 snRNA results in the formation of two populations of nuclear U2 snRNPs. Fully functional U2 snRNPs target IGCs and nascent transcripts. In contrast, incompletely assembled U2 snRNPs accumulate in CBs (Paschedag *et al.*, in revision). In addition, experimental evidence strongly supporting a similar model for the U1 snRNP already exists (Patel *et al.*, 2007). Here, we monitored the fate of newly assembled U5 snRNPs. Capped, fluorescently-labeled U5 snRNAs were synthesized *in vitro* and injected into the cytoplasm of stage IV-V *Xenopus* oocytes. The subnuclear distribution of newly assembled U5 snRNPs was then monitored over time using nuclear spread preparations by immunofluorescence microscopy. CBs were the only structures labeled after 1 hour of incubation, and over time a weaker but specific signal could be detected on nucleoli, IGCs and the LBC loops. After 18 hours of incubation, these structures were readily detectable (although nucleoli consistently displayed the weakest signal) and CBs displayed the highest concentration of U5 snRNPs. (figure 1; magnified views).

The recruitment of U5 snRNP to nascent transcripts does not require U4 or U6 snRNPs . In contrast to the U1 and U2 snRNPs that are recruited to nascent transcripts as individual particles, U5 snRNP is known to further assemble into a U4/U6-U5 tri-snRNP complex prior to its recruitment for spliceosomal assembly. To test how the formation of the tri-snRNP influences the association of U5 snRNPs

with CBs, IGCs and nascent transcripts, we injected fluorescent U5 snRNA into oocytes depleted of U4 and/or U6 snRNAs. Two distinct anti-sense DNA oligonucleotides, U4d and U6f, were used to target the RNase H-mediated degradation of U4 and U6 snRNAs, respectively. Northern blots were performed to monitor the depletion of either U4 or U6 (figure 2). Surprisingly, we found that the absence of the U4/U6 di-snRNP does not affect the sub-nuclear distribution of newly assembled U5 snRNP (figure 2), demonstrating that the formation of the U4/U6.U5 tri-snRNP is not required for the recruitment of U5 snRNP to nascent transcripts. In addition, since the U4/U6.U5 tri-snRNP is required for spliceosomal activity, this result agrees with our previous work demonstrating that the splicing activity present on the LBC loops does not direct the recruitment of the snRNPs, including U5 snRNPs, to nascent transcripts (Patel *et al.*, 2007).

Distinct elements of the U5 snRNP regulate its subnuclear distribution. These data suggested that discrete element(s) of the U5 snRNP acts to license its association with nascent transcripts, IGCs and CBs. To test that hypothesis, we performed a deletion analysis of the U5 snRNA. We used the predicted U5 snRNA secondary structure to guide our analysis, and the various mutants are presented in table 1. Corresponding, fluorescein-labeled RNAs were synthesized and injected into the cytoplasm of stage IV-V Xenopus oocytes, and the subnuclear distributions of the newly formed snRNP complexes were analyzed by fluorescence microscopy on fixed nuclear spreads (figure 3). We found that none of the deletions downstream of the Sm binding site had an effect on the intranuclear trafficking of the newly assembled U5 snRNPs. In fact, figure 3 shows that U5Δ3'96 snRNP, which

is missing stem-loop 2 (SL2), still targeted CBs, IGCs and LBC loops. In contrast, the deletion of the first 47 nucleotides completely inhibited the association of U5Δ5'47 snRNPs with IGCs and nascent transcripts. Interestingly, the removal of the first 47 nucleotides disrupts three predicted structures, stem-loop 1 (SL1), internal loop 1 (IL1) and internal loop 2 (IL2). These structures were then deleted individually (table 1), and we found that while the removal of SL1 or IL2 had no effect, the deletion of IL1 was sufficient to abolish the interaction of U5ΔIL1 snRNPs with IGCs and LBCs (figure 3). Collectively, these data establish IL1 as the element of the U5 snRNP required for its association with nascent transcripts. In addition, as it is the case for U1 and U2 snRNPs, a deletion of most of the nucleotides upstream or downstream of the Sm site does not prevent U5 snRNPs from accumulating within CBs (figure 3 and table 1). It is likely then, that the Sm site corresponds to the element responsible for targeting U5 snRNP to CBs.

Discussion

While the biogenesis of the spliceosomal snRNPs is well documented (reviewed in Patel and Bellini, 2008), the mechanisms that regulate their targeting to nascent transcripts still remain unclear. The current view is that snRNPs are recruited in a step-wise manner for the formation of the spliceosome on target pre-mRNAs. Recent findings, however, indicate that the recruitment of some snRNPs to nascent transcripts can occur in absence of spliceosomal assembly (Patel *et al.*, 2007; Spiluttini *et al.*, 2010; Paschedag *et al.*, in revision). This conclusion stems from two main observations. First, the recruitment of snRNPs is unaffected by the absence of U2 snRNP, which is critical for proper spliceosomal assembly and activity; Second, a non-functional snRNP that cannot comprise part of the spliceosome still associates with nascent transcripts. These data strongly suggest that distinct populations of snRNPs could co-exist on nascent transcripts. One possibility is that snRNPs are initially recruited to nascent transcripts, perhaps as a staging event to increase their local concentrations and allow efficient spliceosome formation upon the emergence during transcription of the necessary cis-acting RNA elements. Another possibility is that snRNPs may function in roles other than splicing. Interestingly, U1 snRNP was recently shown to protect nascent transcripts from premature cleavage and polyadenylation independently of splicing (Kaida *et al.*, 2010; Berg *et al.*, 2012).

Importantly, then, these new insights emphasize the need to examine the regulatory processes by which the splicesomal snRNPs are recruited to nascent transcripts *in vivo*. To our knowledge, LBCs are the only chromosomes where individual

transcription units are readily visible by light microscopy, and we used them in this study to determine the elements of the U5 snRNP that are required for its association with RNAPII nascent transcripts. We previously demonstrated that the recruitment to nascent transcripts of U1 and U2 snRNPs is controlled by discrete elements of their respective RNA moieties: stem-loop 1 of U1 snRNA (Patel *et al.*, 2007) and stem-loops 3 and 4 of U2 snRNA (Paschedag *et al.* in revision). Similarly, our deletion analysis of the U5 snRNP indicates that IL1 is required for its association with the LBC loops and IGCs. Since the U4/U6.U5 tri-snRNP is preassembled prior to integrating the spliceosome, IL1 may act by promoting or stabilizing the association of the U5 snRNP with the U4/U6 di-snRNP. However, this possibility is directly ruled out by the fact that newly assembled U5 snRNPs still target nascent transcripts in oocytes depleted of the U4/U6 di-snRNP. In fact, we find that the intranuclear distribution of newly formed U5 snRNPs is unaffected by the depletion of the U4/U6 di-snRNP; this result agrees with a previous work showing that U5 targets nucleoli as a mono-snRNP (Gerbi *et al.*, 2003b). Thus, IL1 is likely to be a modular element of U5 snRNA that directly controls its intranuclear trafficking, presumably by acting through U5-associated proteins. Interestingly then, U5 snRNP may also be the factor that targets the U4/U6.U5 tri-snRNP to nascent transcripts and IGCs (as suggested in figure 4). Another interpretation is that the U4/U6.U5 tri-snRNP may assemble directly on transcripts after an initially recruitment of U5 snRNP and the U4/U6 di-snRNP but prior to spliceosomal assembly. We are currently testing whether the intranuclear trafficking of the U4/U6 di-snRNP is affected in oocytes lacking the U5 snRNP. Unfortunately, this

work is being hindered by the fact that U5 snRNA could only be partially degraded using an antisense oligonucleotide strategy.

While IGCs and LBC loops are sites targeted by fully assembled snRNPs, CBs and nucleoli are often described as nuclear domains involved in the maturation and/or assembly of snRNPs (Gall *et al.*, 1999; Yu *et al.*, 2001; Gall, 2003; Gerbi *et al.*, 2003a; Matera, 2003). In particular, CBs were directly implicated in the internal modification of the snRNAs by pseudouridylation and 2'-O-methylation (Darzacq *et al.*, 2002; Jady *et al.*, 2003). Here, we find that CBs accumulate newly formed wild-type and mutant U5 snRNP at high concentrations. Interestingly, Gall *et al*. used in situ hybridizations on nuclear spread preparations more than a decade ago to reveal that endogenous U5 snRNPs, as well as all the other spliceosomal snRNPs, are present at a very low concentration in the oocyte CBs (Gall *et al.*, 1999). We recently showed that the newly formed fluorescent U2 snRNPs that accumulate in CBs are incompletely assembled and lack the proteins that are required for targeting nascent transcripts (Paschedag *et al.*, in revision). Similarly, the endogenous U1 snRNP rapidly accumulates in CBs upon truncation of the first stem-loop structure (U1SL1) of the U1 snRNA (Gall *et al.*, 1999), which was subsequently shown to be both necessary and sufficient for targeting U1 snRNP to IGCs and LBC loops (Patel *et al.*, 2007). It is likely then that the newly formed U5 snRNPs accumulated in CBs correspond primarily to incomplete RNP complexes. In addition, since the U5 snRNP comprises part of the tri-snRNP, a stoichiometric imbalance resulting from injecting fluorescent U5 snRNAs into oocytes may also contribute to the accumulation of fluorescent U5 snRNPs in CBs. Indeed, CBs are nuclear bodies

where the formation of the U4/U6.U5 tri-snRNP is enhanced (Stanek *et al.*, 2003; Schaffert *et al.*, 2004; Stanek and Neugebauer, 2004), and one could speculate that monomeric U5 snRNP remains dynamically associated with CBs until assembly of the U4/U6.U5 tri-snRNP.

We previously demonstrated that and U4 and U5 snRNPs target LBC loops in absence of spliceosomal assembly (Patel *et al.*, 2007). Here, we present several pieces of data that further indicate a definite separation between elements controlling intranuclear trafficking and those involved in catalyzing pre-mRNA splicing. First, the deletion of SL1 has not effect on the intranuclear distribution of U5 snRNPs, at least qualitatively. In fact, U5ΔSL1 snRNP targets nascent transcripts and IGCs as efficiently as the wild-type U5 snRNP despite SL1 being essential for the splicing activity of the U5 snRNP; the deletion of only two nucleotides within the SL1 loop is sufficient to inhibit the second trans-esterification reaction of pre-mRNA splicing *in vitro* (O'Keefe and Newman, 1998). Next, the splicing activity of U5 snRNAs lacking IL2 is severely hindered *in vitro* (Dix *et al.*, 1998). Yet, we show here that the deletion of IL2 does not prevent the recruitment of U5 snRNPs to nascent transcripts. Finally, U5 snRNAs lacking IL1, which we show here as being unable to associate with nascent transcripts, are still able to catalyze splicing *in vitro*, albeit with a much lower efficiency that the wild-type U5 snRNA (Dix *et al.*, 1998). Collectively, these data demonstrate a functional modularity of the U5 snRNP, and an uncoupling of the elements that regulate its catalytic activity and its intranuclear trafficking.

Acknowledgments

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Figure legends:

Figure 1. ***Newly assembled U5 snRNPs associate with CBs, IGCs and LBC loops.*** Fluorescently-labeled U5 snRNAs were injected into the cytoplasm of oocytes and nuclear spreads were prepared 18 hours later. Newly formed snRNPs were rapidly targeted to IGCs (asterisks), nascent transcripts and CBs (arrows) where they accumulated at a very high concentration (magnified views). A weaker, but specific signal was also observed within nucleoli (arrow heads).

Figure 2. ***U5 snRNPs can associate with nascent transcripts in absence of U4 and U6 snRNAs .*** Fluorescently-labeled U5 snRNAs were injected into the cytoplasm of oocytes depleted of U4 or/and U6 snRNAs, and nuclear spreads were prepared 18 hours later. Newly formed U5 snRNPs associate with LBC loops and IGCs as well as when U4/U6 snRNAs are present in the oocyte. Northern blots show efficient depletion of U4 and U6 snRNAs induced by oligonucleotide-targeted RNase H activity, while the level of U5 snRNA remains constant.

Figure 3*. The internal loop 1 (IL1) is critical for targeting U5 snRNP to nascent transcripts and IGCs.* Fluorescently labeled, full-length U5 snRNA and various mutants were injected into stage IV-V oocytes. Nuclear spreads were prepared 18 hours later and the subnuclear distributions of newly formed U5 snRNPs were analyzed by immunofluorescence microscopy. Fluorescent micrographs show a

merged image of the fluorescent RNAs (green) and the DNA (red). In the schematics of U5 snRNA mutants, deletions are shown as red lines.

Figure 4. ***Model of U5 snRNP intranuclear trafficking.*** After injection in the cytoplasm, fluorescent U5 snRNAs associate with a heteroheptameric ring of Sm proteins, which is sufficient upon nuclear entry for the association of newly formed U5 snRNPs with CBs. The interaction with CBs is dynamic, and further modification/assembly of U5 snRNP and formation of the U4/U6.U5 tri-snRNP can occur in and out of CBs. The IL1 structure (shown here in blue lines) of U5 snRNA, most likely bound by U5-specific proteins, is the element that eventually targets the U5 snRNP, and possibly the U4/U6.U5 tri-snRNP, to IGCs and nascent transcripts (LBC loops).

Table I. Localization of wild-type and mutant U5 snRNPs as defined on nuclear spreads.

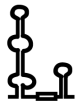
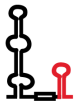
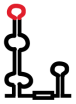
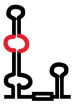
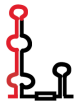
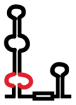
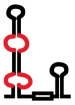
Injected snRNAs	Intranuclear localization			
	LBC loops	IGCs	CBs	Nucleoli
 U5 WT	+	+	+	+/-
 U5Δ3'96				
 U5ΔSL1				
 U5ΔIL2				
 U5Δ5'47	-	-	+	+/-
 U5Δ5'68				
 U5ΔIL1				
 U5ΔIL1+IL2				

TABLE I

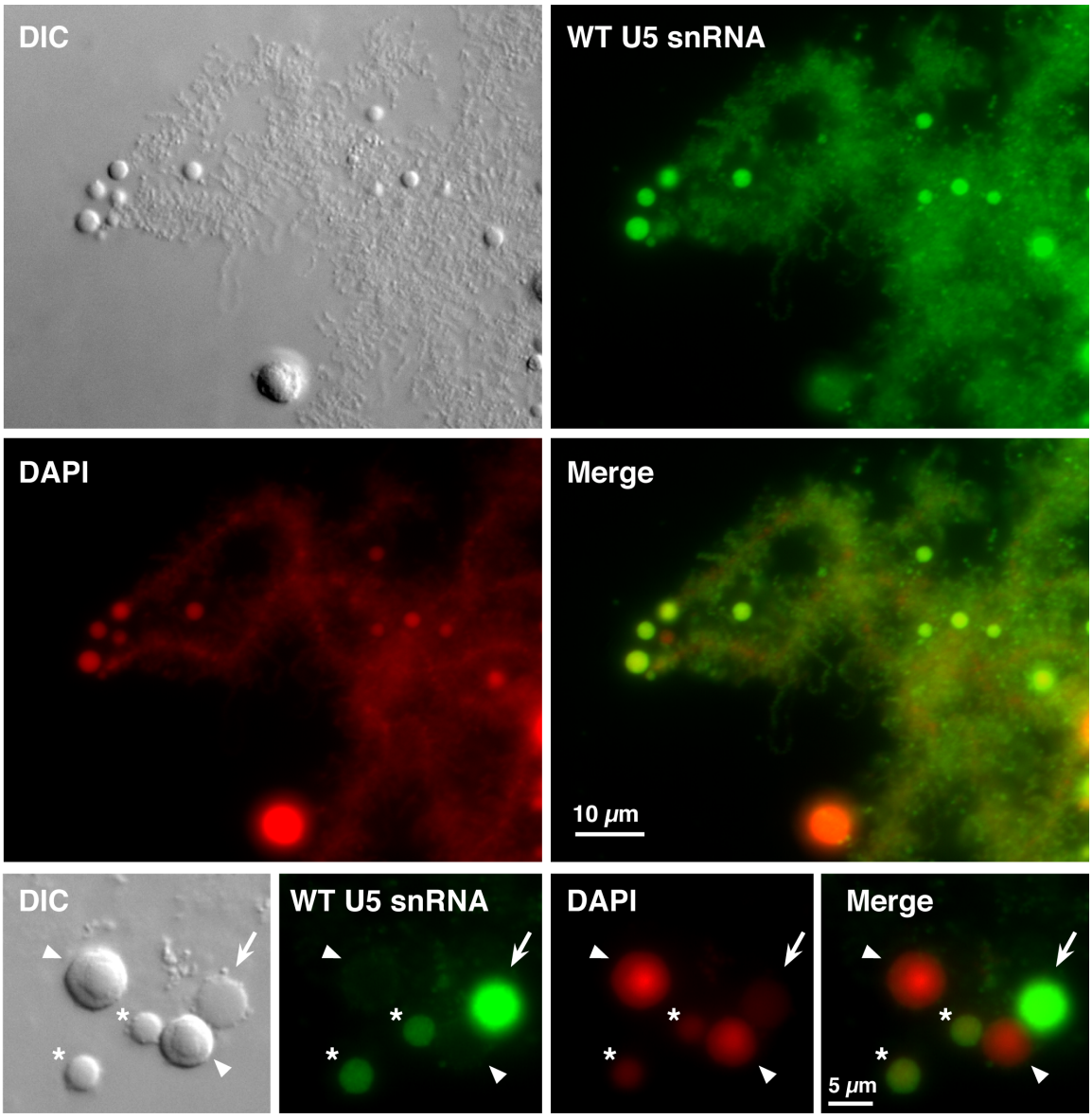


FIGURE 1

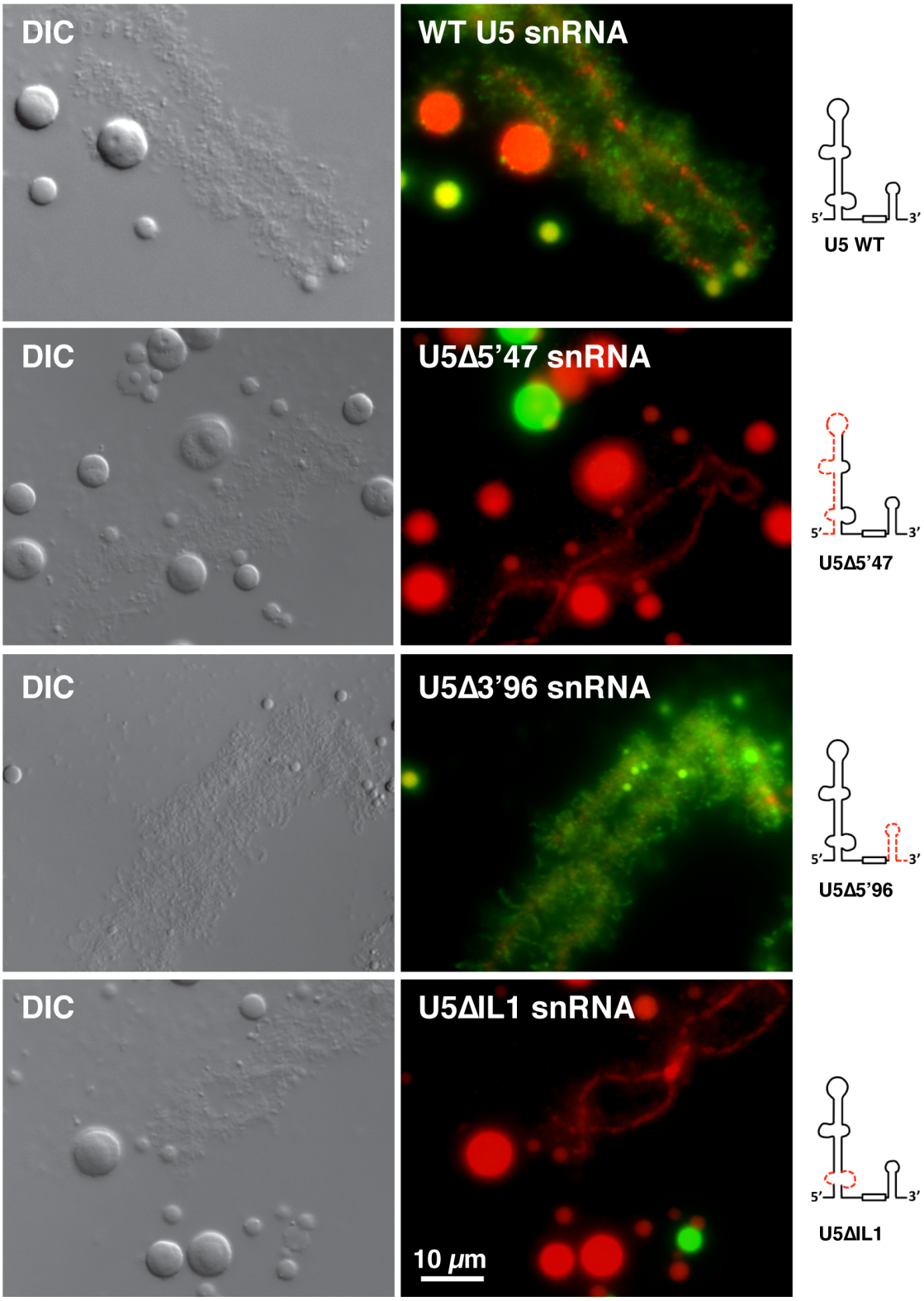

DIC
WT U5 snRNA
5′
3′
U5 WT
DIC
U5Δ5′47 snRNA
5′
3′
U5Δ5′47
DIC
U5Δ3′96 snRNA
5′
3′
U5Δ5′96
DIC
U5ΔIL1 snRNA
10 μm
5′
3′
U5ΔIL1

FIGURE 2

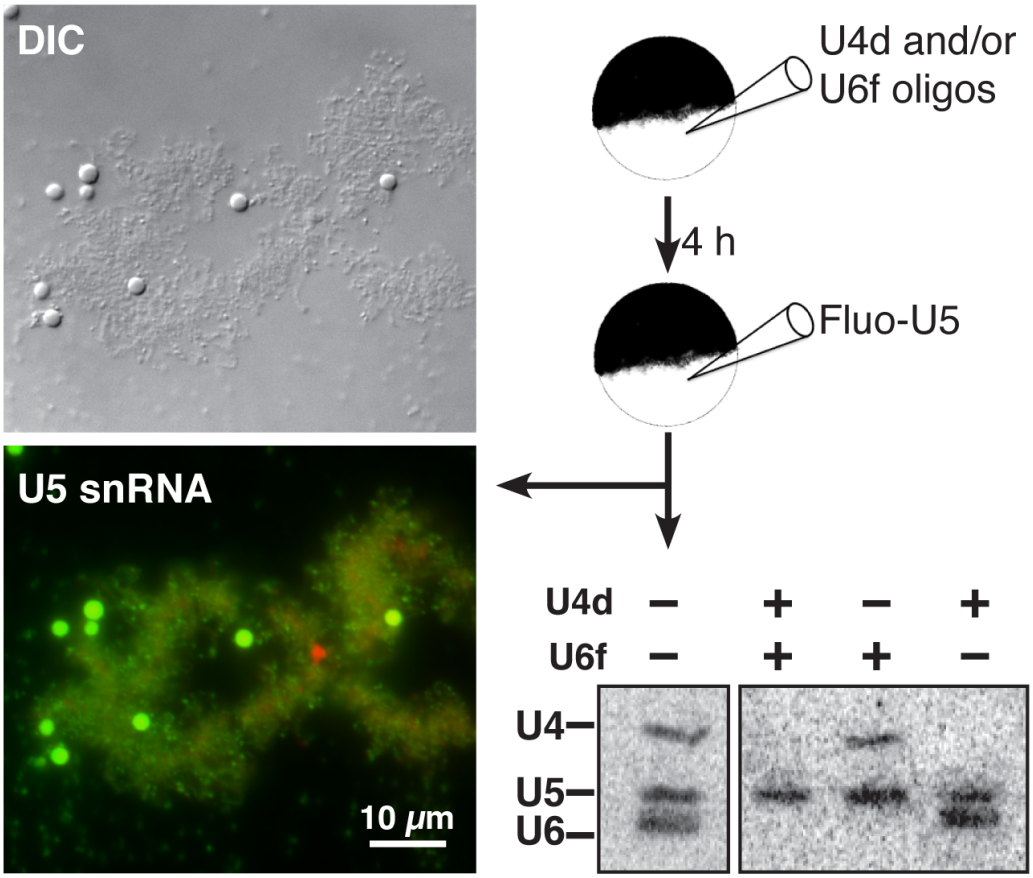

DIC
U5 snRNA
10 μm
U4d and/or
U6f oligos
4 h
Fluo-U5
U4d – + – +
U6f – + + –
U4
U5
U6

FIGURE 3

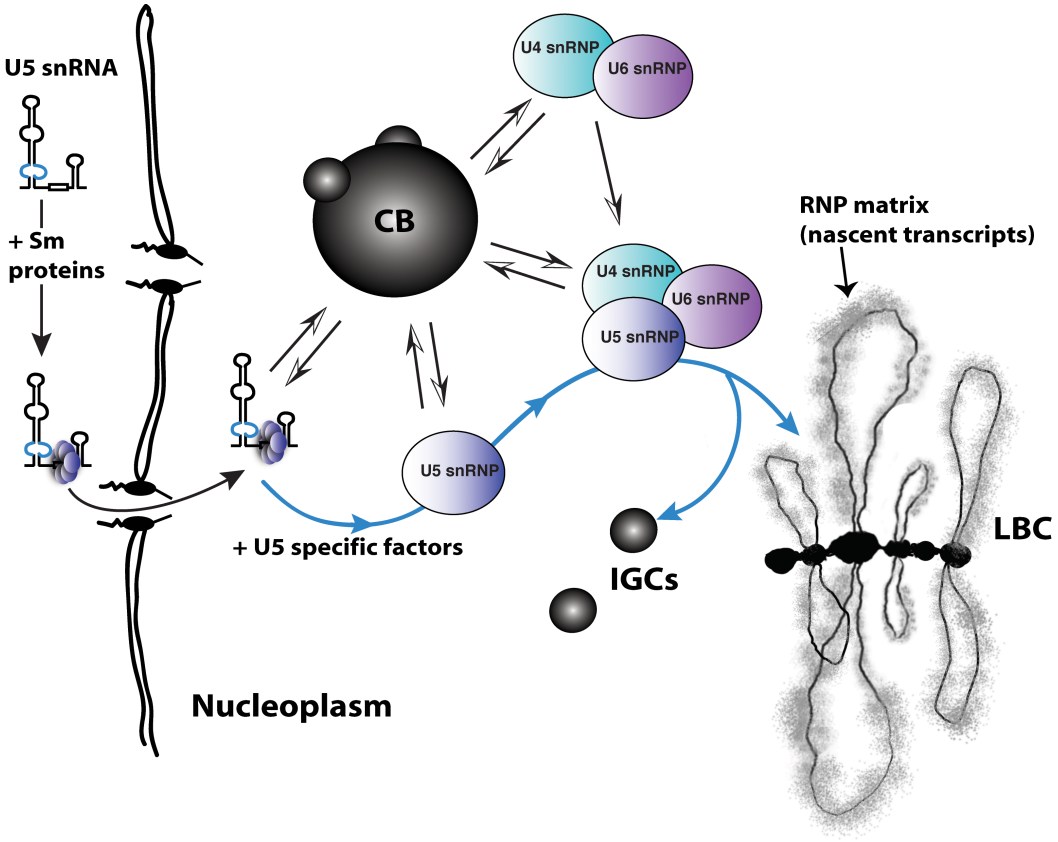

U5 snRNA
+ Sm
proteins
U4 snRNP
U6 snRNP
CB
U4 snRNP
U6 snRNP
U5 snRNP
RNP matrix
(nascent transcripts)
U5 snRNP
+ U5 specific factors
IGCs
LBC
Nucleoplasm

FIGURE 4