Table S1

Transfer rate k	KO (nm/s)	WT (nm/s)	KD (nm/s)	MW (kDa)
U2	-	16 ± 1	17 ± 1	1000
U4	590 ± 170	280 ± 50	270 ± 80	300
U5	-	81 ± 3	70 ± 5	1000
U5_KD	25 ± 1	-	79 ± 5	900
U6	47 ± 1	21 ± 6	26 ± 1	400
U4/U6	0.5 ± 0.3	0.6 ± 0.4	1.2 ± 0.3	540
U4/U6.U5	-	2.9 ± 0.3	3.0 ± 0.5	1800
S3	-	102 ± 50	31 ± 18	90

Transfer rates obtained from global analysis of FRAP data. Coefficients k_{U2}, k_{U5}, k_{U5_h6}, k_{U4}, k_{U6_S3}, k_{S3}, $k_{U4/U6}$, $k_{U4/U6•U5}$ represent apparent transfer rates for diffusion of particles between nucleoplasm and CB. Errors were estimated as standard deviations from multiple experiments and fittings (typically n=10-12). Molecular masses of the snRNP complexes have been estimated previously (Sander *et al.*, 2006; Behzadnia *et al.*, 2007).

Supplementary Video

SuplVideo1 FRAP experiment. HeLa cell line stably expressing hPrp4-GFP after depletion of hPrp6 protein. FRAP was measured 48h after siRNA treatment using the DeltaVision microscope system at 37 °C and in 5% CO_2 atmosphere. GFP was bleached by a 100 ms laser pulse at 488 nm (50% of the laser power level, spot No. 1). Fluorescence recovery was monitored using the *Adaptive time interval* setting (Worx) for 60s. Time is shown in seconds; T = 0 is the time of the bleach. W=525 is the emission wavelength used for fluorescence detection. Bar: 10 μm.

Supplementary Figure

Figure S1 Localization of U2 snRNP. (A) U2A'-GFP is localized to the cell nucleus and to Cajal bodies depicted by coilin immunostaining. Accumulation of U2A'-GFP in Cajal bodies was not altered after hPrp6 knock-down. Bar: 10 μm. (B) Immunoprecipitation of U2A'-GFP showed that the tagged protein is incorporated into the U2 snRNP. Positions of individual snRNAs and rRNAs are indicated. The RNA species depicted by asterisk that consistently immunoprecipitate with U2A' is likely the U11 snRNA.

Supplementary Material

Mathematical description of the model

Dynamics of the proposed model of step-wise U4/U6•U5 tri-snRNP assembly in CB shown in Figure 2A can be described by a system of differential equations written in terms of individual snRNP concentrations inside the CB (indexed *in*) and in the surrounding nucleoplasm (indexed *out*):

$$\frac{d[U4]_{out}}{dt}.\frac{V_{out}}{S} = -k_{U4}[U4]_{out} + k_{U4}[U4]_{in} \tag{S1}$$

$$\frac{d[U4]_{in}}{dt}.\frac{V_{in}}{S} = k_{U4}[U4]_{out} - k_{U4}[U4]_{in} - (k_1[U4]_{in}[U6]_{in.} - k_{-1}[U4/U6]_{in}).\frac{V_{in}}{S} \tag{S2}$$

$$\frac{d[U6]_{out}}{dt}.\frac{V_{out}}{S} = -k_{U6}[U6]_{out} + k_{U6}[U6]_{in} \tag{S3}$$

$$\frac{d[U6]_{in}}{dt}.\frac{V_{in}}{S} = k_{U6}[U6]_{out} - k_{U6}[U6]_{in} - (k_1[U4]_{in}[U6]_{in} - k_{-1}[U4/U6]_{in}).\frac{V_{in}}{S} \tag{S4}$$

$$\frac{d[U5]_{out}}{dt}.\frac{V_{out}}{S} = -k_{U5}[U5]_{out} + k_{U5}[U5]_{in} \tag{S5}$$

$$\frac{d[U5]_{n}}{dt}.\frac{V_{in}}{S} = k_{U5}[U5]_{out} - k_{U5}[U5]_{in} - (k_2[U5]_{in}[U4/U6]_{in} - k_{-2}[U4/U6\bullet U5]_{in}[S3]_{in}).\frac{V_{in}}{S} \tag{S6}$$

$$\frac{d[U4/U6]_{out}}{dt}.\frac{V_{out}}{S} = -k_{U4/U6}[U4/U6]_{out} + k_{U4/U6}[U4/U6]_{in} \tag{S7}$$

$$\frac{d[U4/U6]_{in}}{dt}.\frac{V_{in}}{S} = k_{U4/U6}[U4/U6]_{out} - k_{U4/U6}[U4/U6]_{in} + \\ +(k_1[U4]_{in}[U6]_{in} - k_{-1}[U4/U6]_{in} - k_2[U5]_{in}[U4/U6]_{in} + k_{-2}[U4/U6\bullet U5]_{in}[S3]_{in}).\frac{V_{in}}{S} \tag{S8}$$

$$\frac{d[S3]_{out}}{dt}.\frac{V_{out}}{S} = -k_{S3}[S3]_{out} + k_{S3}[S3]_{in} \tag{S9}$$

$$\frac{d[S3]_{n}}{dt}.\frac{V_{in}}{S} = k_{S3}[S3]_{out} - k_{S3}[S3]_{in} + (k_2[U5]_{in}[U4/U6]_{in} - k_{-2}[U4/U6\bullet U5]_{in}[S3]_{in}).\frac{V_{in}}{S} \tag{S10}$$

$$\frac{d[U4/U6\bullet U5]_{out}}{dt}.\frac{V_{out}}{S} = -k_{U4/U6\bullet U5}[U4/U6\bullet U5]_{out} + k_{U4/U6\bullet U5}[U4/U6\bullet U5]_{in} \tag{S11}$$

$$\frac{d[U4/U6\bullet U5]_{in}}{dt}.\frac{V_{in}}{S} = k_{U4/U6\bullet U5}[U4/U6\bullet U5]_{out} - k_{U4/U6\bullet U5}[U4/U6\bullet U5]_{in} + \\ +(k_2[U5]_{in}[U4/U6]_{in} - k_{-2}[U4/U6\bullet U5]_{in}[S3]_{in}).\frac{V_{in}}{S} \tag{S12}$$

Terms V_{in} and V_{out} stand for volume of CB and nucleoplasm, respectively, S is a surface of the CB. For definition of rate constants and individual kinetic species see Figure 2A.

U2 snRNP dynamics is independent on hPrp6

In order to further test whether the effect of hPrp6 depletion was specific for tri-snRNP components, compartmental analysis of U2A'-GFP fluorescence recoveries before and after the siRNA treatment (hPrp6 knock-down) was performed: We described the U2 snRNP exchange with a simple model comprising the transfer rate k_{U2} and the rate of a hypothetical interaction of U2 snRNP with another unidentified compound X within the CB, k_3 (Eq. S13, S14). Without this interaction we could not explain observed accumulation of U2 in CBs (Supplementary Figure S1A) since concentrations of U2 inside and outside of the CB would equilibrate to the same value.

$$\frac{d[U2]_{out}}{dt}.\frac{V_{out}}{S} = -k_{U2}[U2]_{out} + k_{U2}[U2]_{in} \tag{S13}$$

$$\frac{d[U2]_{in}}{dt}.\frac{V_{in}}{S} = k_{U2}[U2]_{out} - k_{U2}[U2]_{in} - \left[k_3[U2]_{in}[X_{in}] - k_{-3}[U2/X]_{in}\right]\frac{V_{in}}{S} \tag{S14}$$

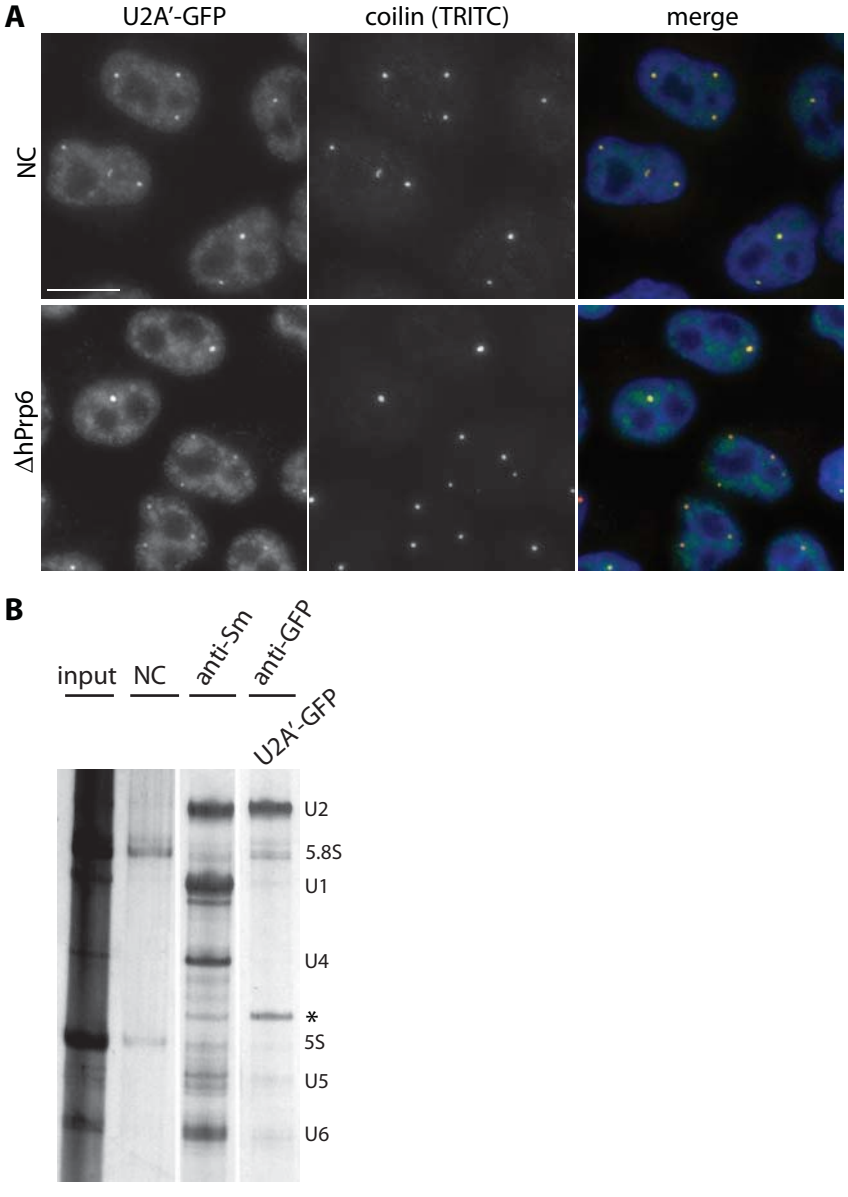
After the analysis of data from mock treated cells (WT) and cells after the hPrp6 knock-down (KD) we obtained almost identical values of the transfer rate k_{U2}, k_{U2}(WT) = 16 ± 1 nm.s^{-1}, and k_{U2}(KD) = 17 ± 1 nm.s^{-1}. We concluded that the hPrp6 knock-down had a specific effect on tri-snRNP formation.

Supplementary references:

Behzadnia, N., Golas, M.M., Hartmuth, K., Sander, B., Kastner, B., Deckert, J., Dube, P., Will, C.L., Urlaub, H., Stark, H., and Luhrmann, R. (2007). Composition and three-dimensional EM structure of double affinity-purified, human prespliceosomal A complexes. EMBO J *26*, 1737-1748.

Sander, B., Golas, M.M., Makarov, E.M., Brahms, H., Kastner, B., Luhrmann, R., and Stark, H. (2006). Organization of core spliceosomal components U5 snRNA loop I and U4/U6 Di-snRNP within U4/U6.U5 Tri-snRNP as revealed by electron cryomicroscopy. Mol Cell *24*, 267-278.

Figure S1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coilin (TRITC)
merge
NC
ΔhPrp6
B
input
NC
anti-Sm
anti-GFP
U2A'-GFP
U2
5.8S
U1
U4
*
5S
U5
U6