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Human U1 snRNA forms a new chromatin-associated snRNP with TAF15

Laure Jobert[1], Natalia Pinzón[2]*, Elodie Van Herreweghe[2], Beáta E. Jády[2], Apostolia Guialis[3], Tamás Kiss[2]+ & László Tora[1]++*

[1]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Department of Functional Genomics, CNRS UMR 7104, INSERM U 964, Université de Strasbourg, Illkirch, France, [2]Laboratoire de Biologie Moléculaire Eucaryote, Université Paul Sabatier, Toulouse, France, and [3]National Hellenic Research Foundation, Athens, Greece

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The U1 small nuclear RNA (snRNA)—in the form of the U1 spliceosomal Sm small nuclear ribonucleoprotein particle (snRNP) that contains seven Sm and three U1-specific RNP proteins—has a crucial function in the recognition and removal of pre-messenger RNA introns. Here, we show that a fraction of human U1 snRNA specifically associates with the nuclear RNA-binding protein TBP-associated factor 15 (TAF15). We show that none of the known protein components of the spliceosomal U1-Sm snRNP interacts with the newly identified U1-TAF15 snRNP. In addition, the U1-TAF15 snRNP tightly associates with chromatin in an RNA-dependent manner and accumulates in nucleolar caps upon transcriptional inhibition. The Sm-binding motif of U1 snRNA is essential for the biogenesis of both U1-Sm and U1-TAF15 snRNPs, suggesting that the U1-TAF15 particle is produced by remodelling of the U1-Sm snRNP. A demonstration that human U1 snRNA forms at least two structurally distinct snRNPs supports the idea that the U1 snRNA has many nuclear functions.

Keywords: $TAF_{II}68$; non-coding RNA; U1-70K

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[1]Institut de Génétique et de Biologie Moléculaire et Cellulaire, Department of Functional Genomics, CNRS UMR 7104, INSERM U 964, Université de Strasbourg, 1 Rue Laurent Fries, BP 10142, 67404 Illkirch, France
[2]Laboratoire de Biologie Moléculaire Eucaryote, Université Paul Sabatier, UMR 5099, IFR 109, 118 Route de Narbonne, 31062 Toulouse, France
[3]National Hellenic Research Foundation, 48 Vas. Constantinou Avenue, 11635 Athens, Greece
*These authors contributed equally to this work
+Corresponding author. Tel: +33 561 335 907, Fax: +33 561 335 886:
E-mail: tamas@ibcg.biotoul.fr
++Corresponding author. Tel: +33 388 653 444, Fax: +33 388 653 201;
E-mail: laszlo@igbmc.u-strasbg.fr

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INTRODUCTION

The U1 spliceosomal Sm small nuclear ribonucleoprotein particle (snRNP), which is composed of the 164-nucleotide-long U1 small nuclear RNA (snRNA), seven Sm proteins (B, D1, D2, D3, E, F and G) and three U1-specific (U1-70K, U1-A and U1-C) snRNP proteins, together with the U2, U4, U5 and U6 snRNPs, and many dozens of protein factors, constitutes the spliceosome that is responsible for the removal of pre-messenger RNA (pre-mRNA) introns (Will & Lührmann, 2006). At the early stage of spliceosome assembly, the U1 snRNP associates with the 5′ splice site through RNA–RNA and protein–RNA interactions, committing the nascent pre-mRNA to the splicing pathway. The U1 snRNP/snRNA has also been implicated in the enhancement of transcription initiation by RNA polymerase II (Pol II; Kwek *et al*, 2002; Damgaard *et al*, 2008), stabilization of nascent pre-mRNAs (Hicks *et al*, 2006) and control of viral pre-mRNA polyadenylation (Gunderson *et al*, 1998).

Biogenesis of Sm snRNPs is a complex process (Kiss, 2004). After synthesis by Pol II, the precursor snRNAs (pre-snRNAs) are exported to the cytoplasm, where their assembly with the seven Sm core proteins is promoted by the survival of motor neurons (SMNs) complex. Binding of Sm proteins is essential for both hypermethylation of the primary monomethyl-G cap to trimethyl-G (TMG) and removal of the 3′ tail sequences of pre-snRNAs. Finally, the nascent Sm snRNPs are imported to the nuclear Cajal bodies where their snRNA components undergo site-specific nucleotide modifications (Jady *et al*, 2003).

During the initiation of Pol II transcription, the general Pol II transcription factor IID (TFIID) is the first general transcription factor that recognizes promoter sequences and initiates assembly of the preinitiation complex. TFIID is composed of the TATA-binding protein (TBP) and a series of TBP-associated factors (TAFs). TAF15, formerly known as $TAF_{II}68$, has been identified as a TAF that interacts with a distinct population of TFIID (Bertolotti *et al*, 1996). In addition to a transcriptional activation domain,

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TAF15 also contains an RNA recognition motif (RRM) and many Arg-Gly-Gly repeat motifs, suggesting that it functions as an RNA-binding protein. TAF15 and the structurally related EWS (Ewing sarcoma) and TLS (translocated in liposarcoma) constitute the TET family of proteins. In human sarcomas, translocation of *TET* genes frequently results in chimaeric oncoproteins (Law *et al*, 2006). The TET proteins have been suggested to have a function in regulating transcription (Bertolotti *et al*, 1996, 1999; Jobert *et al*, 2009) or in the splicing of pre-mRNA (Law *et al*, 2006, and references therein), but their precise function remains elusive.

RESULTS AND DISCUSSION

TAF15 forms a new snRNP with U1 snRNA

To detect cellular RNA partners for TAF15, it was immunoprecipitated from a HeLa nuclear extract with two different TAF15 antibodies, and the co-precipitated RNAs were terminally labelled and size-fractionated on a denaturing gel (Fig 1A). Both RNA samples recovered by the TAF15 antibodies were highly enriched by an approximately 165-nucleotide-long RNA that was hardly detectable in control immunoprecipitations. The efficient immunoprecipitation of TAF15 was confirmed by Western blot. When labelled nuclear RNA was run in parallel, it became apparent that the most abundant TAF15-associated RNA co-migrated with U1 snRNA.

To determine unequivocally the identity of the newly detected TAF15-associated RNA, RNase A/T1 protection analysis was carried out by using an RNA probe complementary to the predominant U1A sequence variant of the human U1 snRNA (Fig 1B). RNA co-immunoprecipitated with TAF15 protected the U1A probe, but failed to protect RNA probes specific for the U2 and U4 snRNAs. None of the RNA probes was efficiently protected by mock-immunoprecipitated RNAs. An RNA-immunoprecipitation assay confirmed the *in vivo* association of TAF15 with U1 (supplementary Fig 1 online). Thus, we conclude that human U1 snRNA specifically associates with TAF15.

Many RNA-binding proteins, including U1-70K and TAF15, share an RRM that contains the conserved eight- and six-residue RNP1 and RNP2 motifs. The first two amino acids (Arg 143 and Gly 144) of the RNP1 motif of U1-70K have been shown to be fundamental for binding to U1 snRNA (Surowy, 1989). To test whether the RRM of TAF15 is important for *in vivo* binding to U1, the first and second residues (Lys 280 and Gly 281) in the RNP1 motif of TAF15 were replaced by proline (P) and serine (S), respectively. The resulting TAF15 K280P and G281S mutants, as well as the wild-type TAF15, were transiently expressed as Flag-tagged proteins in HeLa cells. Following anti-Flag immunoprecipitations, the recovery of U1 snRNA was monitored by reverse transcription–quantitative PCR (RT–qPCR; Fig 1C). As compared with the wild-type Flag–TAF15, the mutant Flag–TAF15 K280P and Flag–TAF15 G281S proteins showed about 54% and 64% reduced U1-binding capacities, respectively, indicating that the RNP1 motif contributes to the U1-binding capacity of TAF15.

To determine whether TAF15 binds to a fraction of the U1-Sm snRNP or whether it is a component of a new, not yet identified minor U1 snRNP, co-immunoprecipitation experiments were performed (Fig 1D). Although immunoprecipitation of TAF15 efficiently recovered U1 snRNA, it failed to pull down detectable

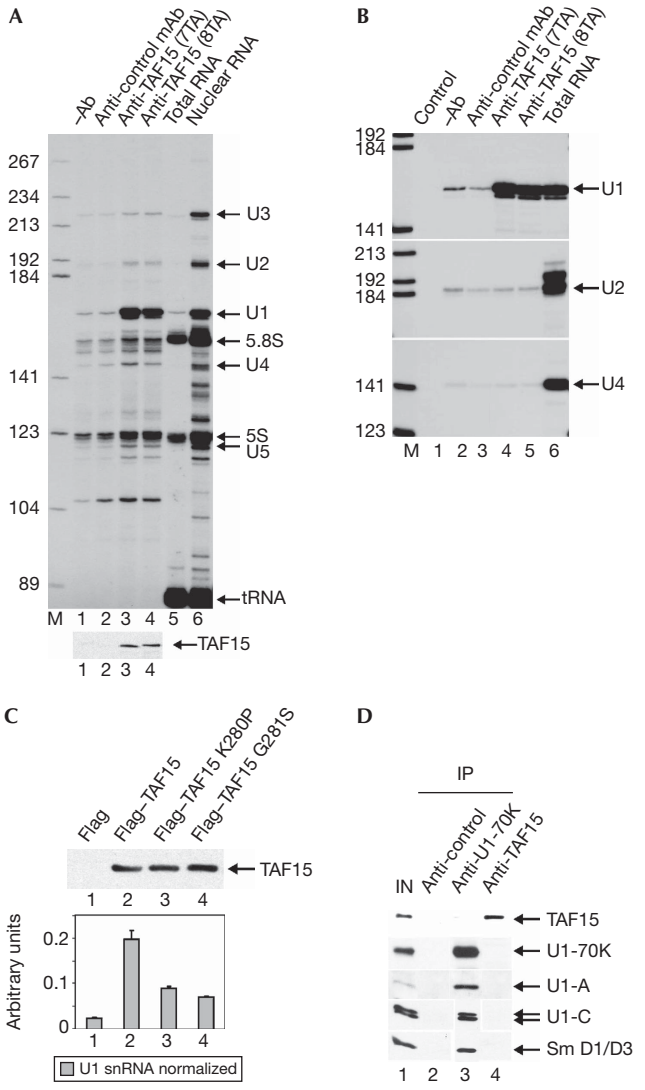

Fig 1 | TAF15 forms a new snRNP with U1 snRNA. (**A**) Immunoprecipitations (IP) were performed from a HeLa nuclear extract with two TAF15 antibodies (lanes 3 and 4), a control antibody (lane 2) or with protein G-Sepharose alone (lane 1). RNAs co-precipitated with TAF15, HeLa total and nuclear RNAs were terminally labelled and separated on a sequencing gel (upper panel). TAF15 immunoprecipitation was analysed by Western blot (lower panel). (**B**) RNAs precipitated by TAF15 antibodies, protein G-Sepharose alone (−Ab) or a control antibody were analysed by RNase A/T1 mapping with RNA probes specific for U1, U2 or U4 snRNAs. The protected RNAs were fractionated on a sequencing gel. Lane 1, control mapping with *Escherichia coli* tRNA. (**C**) Transiently expressed Flag, Flag–TAF15, Flag–TAF15 K280P or Flag–TAF15 G281S proteins were immunoprecipitated by Flag antibodies. Co-immunoprecipitation of U1 snRNA was measured by RT–qPCR and normalized with the amount of U1 co-precipitated with Flag–TAF15. (**D**) Immunoprecipitations from a HeLa nuclear extract (IN) were performed with antibodies as indicated. The co-immunoprecipitated proteins were analysed by Western blot. Ab, antibody; M, size marker; mAb, monoclonal antibody; RT–qPCR, reverse transcription–quantitative PCR; snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein particle; TAF, TBP-associated factor 15; tRNA, transfer RNA.

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amounts of the U1-70K, U1-A, U1-C and D1/D3 Sm proteins that are integral components of the U1-Sm snRNP. By contrast, all of the tested U1 snRNP proteins were efficiently recovered by immunoprecipitation performed with a U1-70K antibody. A trace amount of TAF15 detected in the pellet of the anti-U1-70K immunoprecipitation probably derived from a weak cross-reaction of the U1-70K antibody with TAF15. This assumption was confirmed by the fact that U1-70K failed to co-precipitate with a transiently expressed Flag-tagged TAF15 when it was immunoprecipitated with a Flag antibody (supplementary Fig 2 online).

TAF15 associates with the main isoform of U1 snRNA

Although human cells express minor sequence variants of the U1 snRNA (Kyriakopoulou *et al*, 2006), direct RNA sequencing of 3′-end labelled TAF15-associated U1 snRNA has revealed a nucleotide sequence identical to the Gly 41–Gly 164 3′-terminal portion of the most abundant U1A sequence variant of U1 snRNA (data not shown). Consistent with this, the TAF15-bound U1 snRNA efficiently protected the U1A-specific antisense RNA probe (Fig 1B), showing that TAF15 predominantly associates with the abundant U1A snRNA.

The mature U1-Sm snRNA contains an internal 2′-O-ribose-methylated adenosine (A70), two pseudouridines (Ψ5 and Ψ6) and a 5′-terminal TMG cap. To test whether these post-transcriptional modifications are also present in the TAF15-associated U1 snRNA, the U1-TAF15 snRNP and a positive control, the U1-Sm snRNP, were immunoprecipitated from a HeLa nuclear extract with TAF15- and Sm-specific antibodies (Fig 2A). Consistent with the conclusion that TAF15 and Sm proteins form two distinct snRNPs with U1, the pellets of the Sm and TAF15 immunoprecipitation reactions were devoid of TAF15 and Sm proteins, respectively. The U1 snRNA co-precipitated with TAF15 was further analysed by immunoprecipitation with TMG and H-20 (specific for both TMG and monomethyl-G) antibodies (Fig 2B). RNase A/T1 mapping revealed that both antibodies immunoprecipitated U1 snRNA, showing that the TAF15-bound U1 snRNA carries a TMG cap.

The 2′-O-methylation status of A70 in the TAF15-associated U1 snRNA was determined by primer extension analysis in the presence of low dNTP concentration, which is known to stop reverse transcriptase before the methylated nucleotide (Jady *et al*, 2003; Fig 2C). When TAF15- or Sm-associated U1 snRNAs were analysed with a U1-specific primer at low dNTP concentration, a strong stop signal was observed before A70 in both cases, showing that the A70 residue is 2′-O-methylated in both U1 samples.

Pseudouridylation of the U5 and U6 residues was determined by the CMC primer extension method (Fig 2D). CMC reacts with pseudouridines and arrests reverse transcriptase one nucleotide before them (Jady *et al*, 2003). Primer extension analysis of CMC-treated TAF15- and Sm-associated U1 snRNAs resulted in stops one nucleotide before the U5 and U6 residues, showing that these uridines are converted into pseudouridine. In summary, we conclude that the U1 snRNA components of the U1-TAF15 and U1-Sm snRNPs have no distinctive structural characteristics.

Biogenesis of U1-TAF15 snRNP requires the Sm motif of U1

A demonstration that the U1-TAF15 snRNP lacks Sm proteins, but its snRNA component is correctly processed suggests that the TAF15-associated fraction of U1 snRNA follows an

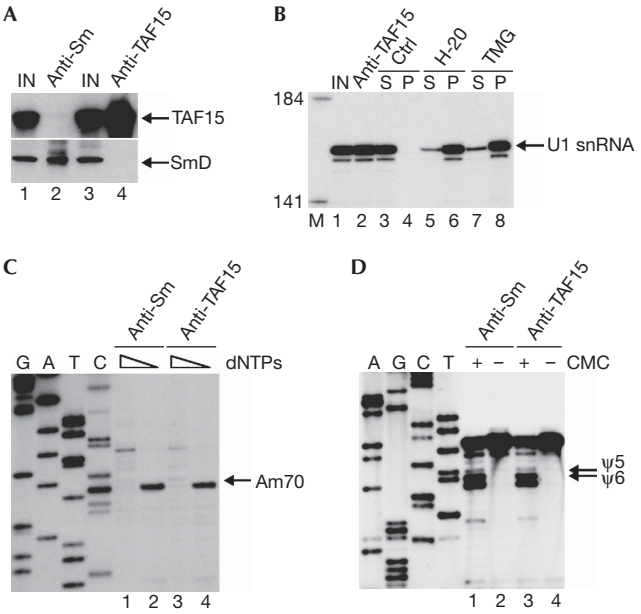

Fig 2 | Characterization of U1 snRNA associated with TAF15. (**A**) HeLa U1-TAF15 and U1-Sm snRNPs were immunoprecipitated from a nuclear extract (IN) with antibodies specific for TAF15 and SmD proteins and analysed by Western blot. (**B**) U1 snRNA co-purified with TAF15 was immunoprecipitated with TMG or H-20 antibodies. Mock immunoprecipitation (Ctrl) was performed with beads alone. RNAs recovered from the supernatant (S) and pellet (P) of each immunoprecipitation were analysed by RNase A/T1 mapping. (**C**) U1 RNAs associated with Sm or TAF15 were analysed by primer extension in the presence of 1 or 0.04 mM dNTPs. (**D**) Sm- or TAF15-associated U1 RNA, either treated (+) or non-treated (−) with CMC, was analysed by primer extension. A, G, C and T: dideoxy sequencing ladders. Am70, methylated adenosine 70; M, size marker; SmD, one of the members of the nine Sm core proteins that bind to snRNAs (U1, U2, U4 and U5); snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein particle; TAF15, TBP-associated factor 15; TMG, trimethyl-G.

'Sm-independent' processing pathway. To test this, a series of mutant U1 snRNAs, U1*sm1* to U1*sm5*, carrying altered Sm-binding motifs were transiently expressed in HeLa cells (Fig 3A). RNase A/T1 mappings revealed a weak accumulation for each mutant U1 RNA. Besides mature-sized U1 RNAs, we detected several 3′-extended unprocessed or partly processed pre-U1 snRNAs, which were also present in non-transfected cells.

To determine the subcellular localization of the weakly expressed mutant U1 RNAs, the U1*sm3* RNA and as a control, an Sm mutant U2 snRNA (U2*sm*), were expressed in HeLa cells (Fig 3B). In contrast to U1*sm3*, only one 3′-extended precursor form of the U2*sm* RNA accumulated. Mapping of cytoplasmic and nuclear RNAs revealed that the U2*sm* RNA and all variants of the U1*sm3* RNA accumulated exclusively in the cytoplasm, indicating that they represent dead-end products of snRNA biogenesis. Consistently, neither Sm nor TAF15 antibodies pulled down U1*sm3* RNAs (Fig 3C). A demonstration that the Sm motif is essential not only for the biogenesis of the U1-Sm snRNP but also for the accumulation of the U1-TAF15 snRNP, strongly supports the idea that U1-TAF15 is produced by remodelling of the U1-Sm

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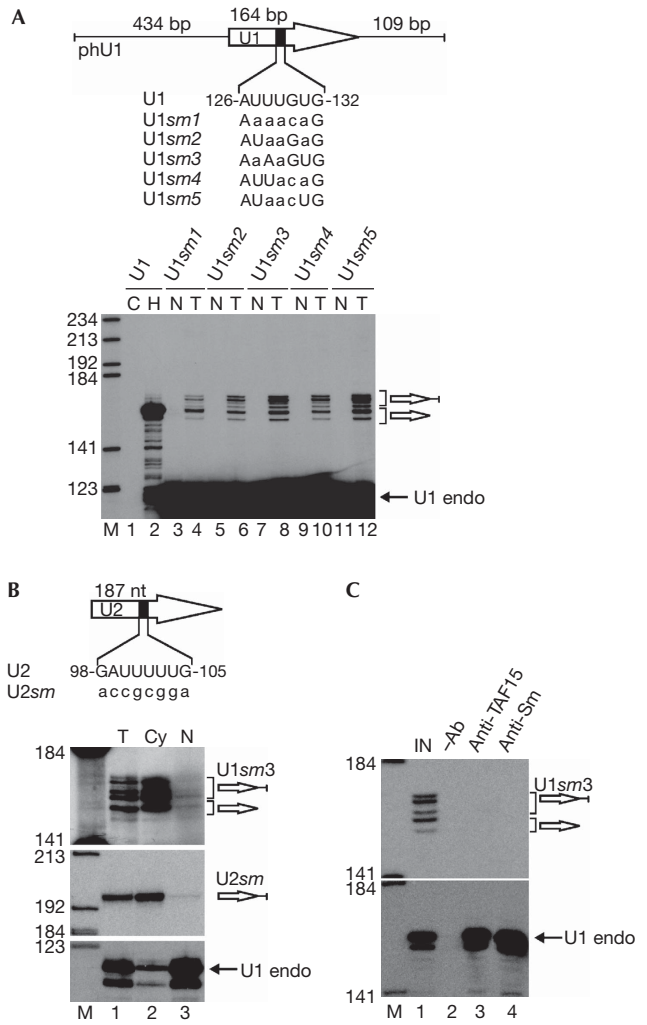

Fig 3 | Biogenesis of U1-TAF15 snRNP requires the U1 Sm motif. (**A**) Accumulation of mutant U1 RNAs (U1*sm1* to U1*sm5*) in transfected (T) and non-transfected (N) HeLa cells was analysed by RNase mapping. Structure of the phU1 expression construct is shown. Mappings with *Escherichia coli* tRNA (C) and HeLa total RNA (H). (**B**) Distribution of transiently expressed U1*sm3* and U2*sm* RNAs in the cytoplasmic (Cy) and nuclear (N) fractions of transfected HeLa cells (T) was determined by RNase mapping. (**C**) Association of U1*sm3* RNA (IN) with TAF15 and Sm proteins was assayed by immunoprecipitation with TAF15 and Sm antibodies followed by RNase mapping. –Ab, control-immunoprecipitation; M, size marker; snRNP, small nuclear ribonucleoprotein particle; TAF15, TBP-associated factor 15; tRNA, transfer RNA.

snRNP after reimportation from the cytoplasm. The cytoplasmic assembly of Sm snRNPs is promoted by the SMN complex that can also facilitate the disassembly of Sm snRNPs (Chari *et al*, 2008). Thus, SMN might participate in the biogenesis of U1-TAF15 snRNP by promoting the disassembly of U1-Sm snRNP. The SMN-dependent biogenesis of U1-TAF15 might take place in the Cajal bodies, as spliceosomal snRNPs repeatedly cycle through Cajal bodies (Stanek *et al*, 2008) and SMN accumulates in these nucleoplasmic organelles (Battle *et al*, 2006).

The U1-TAF15 snRNP associates with chromatin

Cell extracts prepared in the presence of 0.2 M NaCl, which is routinely used to isolate nucleoplasmic snRNPs, contained only trace amounts of TAF15 (data not shown). To examine the possibility that TAF15 and the U1-TAF15 snRNP associate with chromatin, HeLa nuclei were extracted with increasing concentrations of NaCl in the presence or absence of RNase A (Fig 4A). Without RNase, no significant amount of TAF15 was solubilized at 0.2 M or lower salt concentrations. Increasing the salt concentration of the extraction buffer up to 1 M supported the solubilization of only about 50% of nuclear TAF15. Inclusion of RNase A significantly facilitated the solubilization of TAF15 at each step, indicating that TAF15 tightly interacts with chromatin in an RNA-dependent manner.

To analyse further the subcellular distribution of TAF15 and, more importantly, the U1-TAF15 snRNP, HeLa cells were fractionated into cytoplasmic, nucleoplasmic and chromatin fractions (Fig 4B; supplementary information online). Nuclear fractionation was confirmed by the detection of histone H3 in the chromatin extract. TAF15 was not detectable in the cytoplasm, but it was present in the nucleoplasmic fraction eluted with 350 mM salt and in the chromatin extract, where proteins were further solubilized by micrococcal nuclease digestion. To test whether the chromatin-associated fraction of TAF15 binds to U1 snRNA, comparable amounts of TAF15 (after normalization of TAF15 amounts) were immunoprecipitated from the nucleoplasmic and chromatin extracts (Fig 4C). The recovery of U1 snRNA was monitored by RNase mapping and RT–qPCR (Fig 4D). TAF15 immunoprecipitated from the chromatin extract bound about four times more U1 snRNA than did TAF15 derived from the nucleoplasmic extract, indicating that the U1-TAF15 snRNP is highly enriched in the chromatin of HeLa nuclei and that only a fraction of the nuclear soluble form of TAF15 binds to U1 snRNA.

To characterize further the nuclear-soluble and the chromatin-associated U1-TAF15 snRNPs, the nucleoplasmic and chromatin extracts were size-fractionated by chromatography, and fractions were analysed by Western blotting with antibodies specific for TAF15, U1-70K, TBP and TAF5 (Fig 4E; supplementary Fig 4A online). In both extracts, we detected high molecular weight complexes in fractions 14–16, which contained TAF5, TBP and TAF15. When distribution of the U1 snRNA was determined by RT–qPCR and compared with that of U1-70K, it became apparent that the U1-Sm snRNP eluted mainly in fractions 24–26 of both nucleoplasmic and chromatin extracts. In contrast to U1-70K, TAF15 eluted mainly in fractions 30–34 in both extracts, further showing that U1-70K and TAF15 are present in different complexes. When fractions 30–34, obtained by fractionation of nucleoplasmic or chromatin extracts, were pooled and TAF15 was immunoprecipitated, the U1 snRNA was present in both immunoprecipitations, showing that both the nucleoplasmic- and chromatin-soluble forms of TAF15 associate with U1 snRNA (Fig 4F; supplementary Figs 3B and 4C online).

As TAF15 was originally identified as a TFIID-associated protein (Bertolotti *et al*, 1996), we investigated its interaction with two components of TFIID, TBP and TAF5, in the size-fractionated nucleoplasmic- and chromatin-soluble extracts (supplementary Figs 3A and 4B online). In both cases, we found that TAF15 eluted in the low molecular weight fractions (30–34), which contain the U1-TAF15 snRNP, did not associate with TBP

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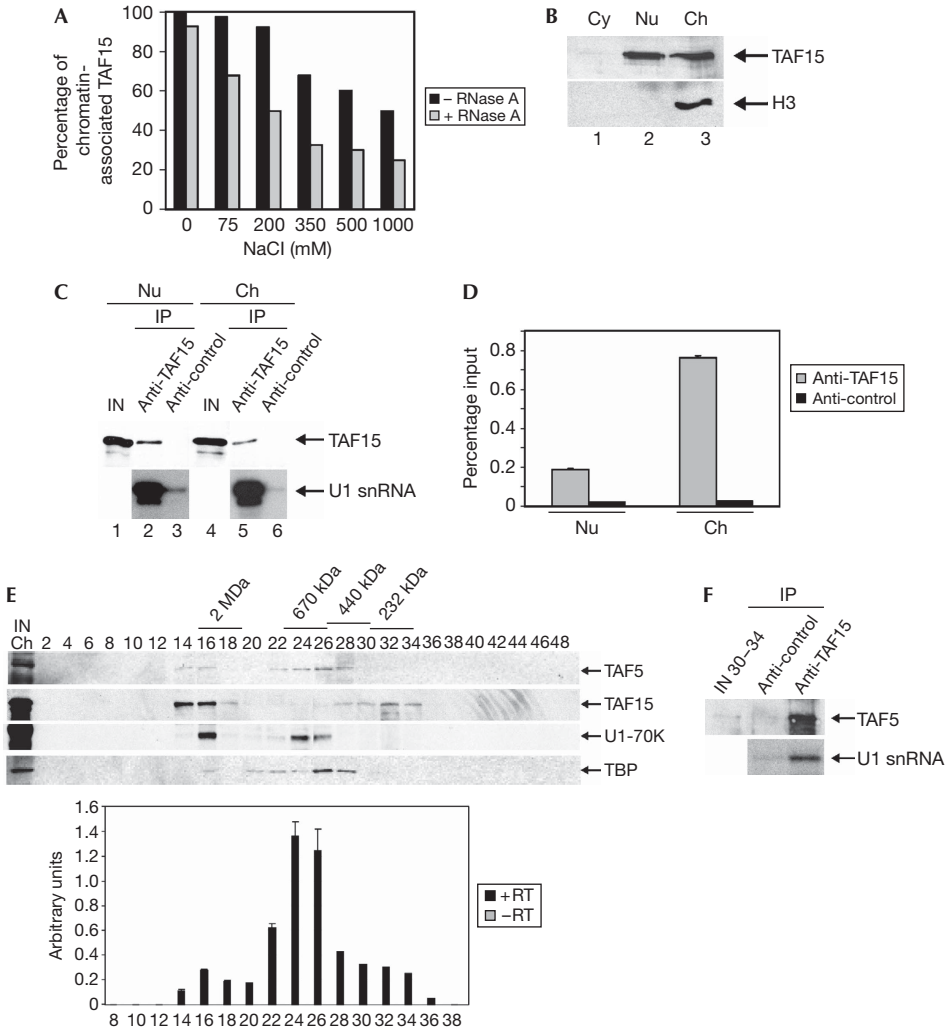

Fig 4 | The U1-TAF15 snRNP associates with chromatin. (**A**) HeLa nuclei were extracted with increasing concentrations of NaCl with or without RNase A. After centrifugation the distribution of TAF15 in the soluble and insoluble fractions was determined by Western blot analysis and densitometric scanning. (**B**) HeLa cell cytoplasmic- (Cy), nucleoplasmic- (Nu) and chromatin (Ch)-soluble fractions were prepared and analysed by Western blot. (**C**) Nucleoplasmic and chromatin fractions were immunoprecipated with TAF15 or control antibodies. Co-precipitation of U1 snRNA was measured by RNase mapping. (**D**) Measurement of the association of TAF15 with U1 by RT–qPCR. (**E**) HeLa chromatin-soluble fraction was fractionated by gel filtration. The input (IN Ch) and even fractions were analysed by Western blot. The distribution of U1 snRNA was analysed by RT–qPCR. Molecular mass markers are indicated. (**F**) Fractions 30–34 from (E) were pooled and subjected to immunoprecipitations with TAF15 or control antibodies. Co-precipitation of U1 snRNA was measured by RNase mapping. IP, immunoprecipitation; RT–qPCR, reverse transcription–quantitative PCR; snRNP, small nuclear ribonucleoprotein particle; TAF15, TBP-associated factor 15; TBP, TATA-binding protein.

and TAF5. By contrast, immunoprecipitation of TAF15 from the large molecular size fractions (14–18) co-precipitated both TAF5 and TBP, but failed to pull down U1 snRNA (supplementary Fig 4C online), indicating that TAF15 interacts with TFIID in a U1-independent manner.

Stress-induced perinucleolar accumulation of U1-TAF15

As both TAF15 and U1 snRNAs have been implicated in Pol II transcription, we investigated the interaction of U1 and TAF15 in transcriptionally arrested HeLa cells (supplementary Fig 5 online). Surprisingly, as compared with TAF15 immunoprecipitated from a control extract, TAF15 immunoprecipitated from the extract of α-amanitin-treated cells showed about 2.5-fold increase in U1 association, indicating that inhibition of Pol II transcription increases the association of TAF15 with U1 snRNA.

Next, we determined the subnuclear localization of the U1-TAF15 snRNP in α-amanitin-treated and control HeLa cells with indirect immunofluorescence and fluorescent *in situ* hybridization (Fig 5). In control cells, both TAF15 and U1 snRNAs localized predominantly to the nucleoplasm, except that U1 showed enrichments in the Cajal bodies and nucleoplasmic speckles (Fig 5A). In cells treated with α-amanitin for 3 h, both TAF15

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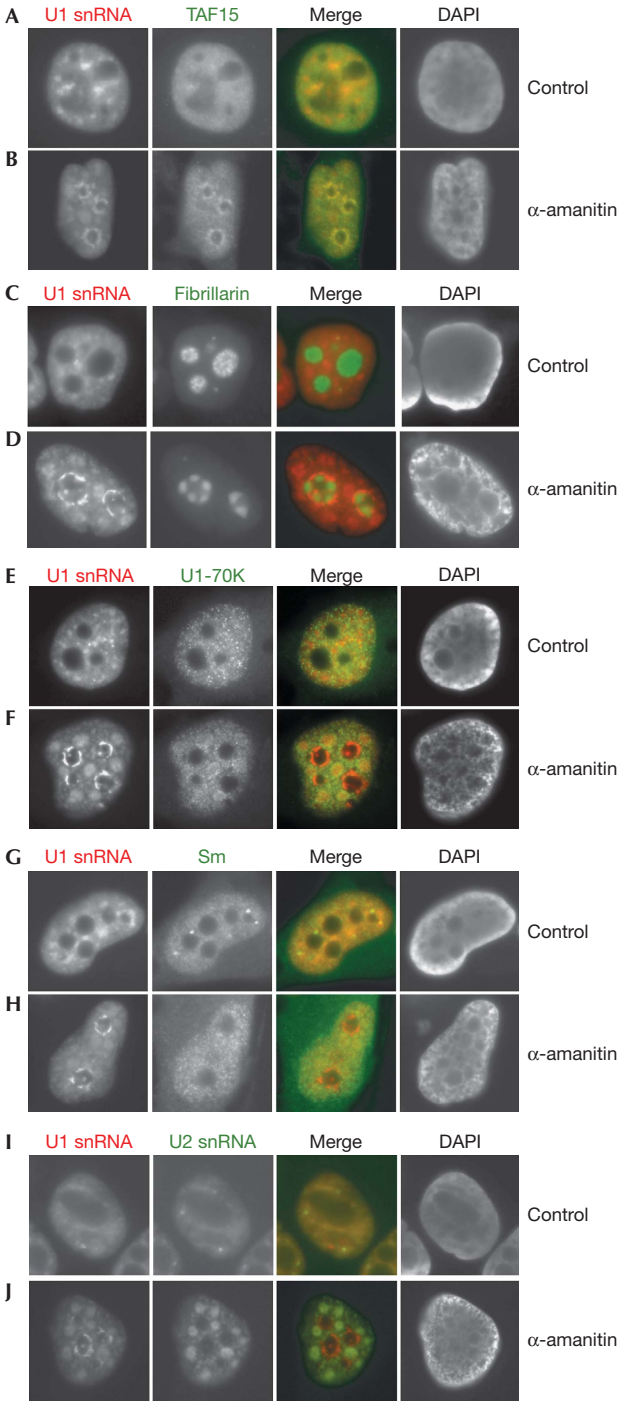

Fig 5 | Transcription-dependent subnuclear localization of U1-TAF15 snRNP. HeLa cells treated either with 20 μg/ml α-amanitin for 3 h or with non-treated (control) were probed with fluorescent oligonucleotides complementary to the human U1 and U2 snRNA, and with antibodies specific for TAF15, U1-70K and Sm proteins. Nucleoli were stained by transient expression of fibrillarin-GFP and DNA was visualized by DAPI staining. DAPI, 4,6-diamidino-2-phenylindole; GFP, green fluorescent protein; snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein particle; TAF15, TBP-associated factor 15.

and U1 were concentrated on the periphery of round-shaped nucleoplasmic domains (Fig 5B). On transient expression of a green fluorescent protein (GFP)-tagged nucleolar protein, fibrillarin, it became apparent that U1 and TAF15 accumulated in perinucleolar cap structures formed on the surface of nucleoli entering transcription inhibition-induced segregation (Fig 5C and D). After completion of nucleolar segregation (5–6 h after α-amanitin administration), the U1-70K snRNP protein is also known to accumulate in the nucleolar caps (Carmo-Fonseca *et al*, 1992; our unpublished data). In contrast to U1 snRNA, neither U1-70K nor Sm snRNP proteins showed perinucleolar accumulation 3 h after α-amanitin administration, excluding the possibility that U1 accumulated in the perinucleolar caps in the form of U1-Sm snRNP (Fig 5E–H). Similarly, the U2-Sm snRNA also failed to concentrate in the nucleolar caps of transcriptionally arrested cells (Fig 5I and J), indicating that the U1-TAF15 snRNP specifically translocates into perinucleolar caps already at an early stage of nucleolar segregation.

The findings that both cellular accumulation and subnuclear distribution of the U1-TAF15 snRNPs are sensitive to the transcriptional activity of the cell might indicate that the U1-TAF15 snRNPs have a Pol II transcription-dependent function. By sequestering U1 snRNA into the U1-TAF15 snRNPs, TAF15 might negatively regulate either the spliceosomal function of the U1-Sm snRNP or the transcription initiation function of the U1–TFIIH complex (Kwek *et al*, 2002). Recently, non-coding RNAs localized to the regulatory regions of transcription units were shown to recruit and modulate the activity of the TET proteins in response to specific signals (Wang *et al*, 2008). Thus, our study might indicate that the inhibition of Pol II transcription increases the amount of chromatin-associated U1-TAF15 snRNP through the recruitment of more TAF15 from the nuclear-'soluble' pool to chromatin. These results might also suggest that the U1-TAF15 snRNP acts following specific signals such as inhibition of Pol II transcription.

In conclusion, we have shown that human U1 snRNA forms at least two structurally and, most likely, functionally distinct snRNP particles. Our findings strongly support the idea that U1 snRNA has many nuclear functions and highlight the intriguing possibility that non-coding RNAs with well-established functions might participate in several cellular processes.

METHODS

Immunoprecipitation and Western blot analysis. Proteins from 500 μg of nuclear extract were immunoprecipitated with 50 μl of protein G-Sepharose (Amersham Pharmacia Biotech, Uppsala, Sweden) and approximately 5–10 μg of the various antibodies as described previously (Bertolotti *et al*, 1996), except that immunoprecipitation buffers containing NaCl instead of KCl were used. Western blot and chemiluminescence detection were performed according to the manufacturer's instructions (Amersham Pharmacia).
Extraction of RNA and analysis of TAF15-bound nucleic acids. RNAs from TAF15 immunoprecipitations were isolated by the guanidinium thiocyanate/phenol–chloroform extraction method. RNA 3′ end labelling with [5′-^{32}P]pCp and T4 RNA ligase (New England BioLabs, Hitchin, UK) and RNase A/T1 protection assay were performed. To generate sequence-specific antisense RNA probes, recombinant pBluescribe plasmids carrying full-length cDNAs of the human U1, U2 or U4 snRNAs were

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linearized and used as templates for *in vitro* transcription with the T7 RNA polymerase (Promega, France) in the presence of [α-^{32}P]CTP (30 Ci/mmol).

Immunofluorescence and *in situ* hybridization. Fixation, permeabilization, immunostaining, *in situ* hybridization of HeLa cells, synthesis and chemical conjugation of amino-modified oligonucleotides with Fluoro-Link Cy3 and Cy5 monofunctional dyes were performed as described at http://singerlab.aecom.yu.edu.

Further experimental procedures are provided in the supplementary information online.

Supplementary information is available at *EMBO reports* online (http://www.emboreports.org).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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