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

Laure Jobert1++, Natalia Pinzón2++, Elodie Van Herreweghe2, Beáta E. Jády2, Apostolia Guialis3, Tamás Kiss2++

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

INTRODUCTION

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: TAF15; non-coding RNA; U1-70K
EMBO reports (2009) 10, 494–500. doi:10.1038/embor.2009.24

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits distribution, and reproduction in any medium, provided the original author and source are credited. This license does not permit commercial exploitation without specific permission.
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-precipitated 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 (Arg143 and Gly144) 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 (Lys280 and Gly281) 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. (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–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.
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.

Pseudouridines 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 'Sm-independent' processing pathway. To test this, a series of mutant U1 snRNAs, U1sm1 to U1sm5, 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 U1sm3 RNA and as a control, an Sm mutant U2 snRNA (U2sm), were expressed in HeLa cells (Fig 3B). In contrast to U1sm3, only one 3'-extended precursor form of the U2sm RNA accumulated. Mapping of cytoplasmic and nuclear RNAs revealed that the U2sm RNA and all variants of the U1sm3 RNA accumulated exclusively in the cytoplasm, indicating that they represent dead-end products of snRNA biogenesis. Consistently, neither Sm nor TAF15 antibodies pulled down U1sm3 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 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...
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 TFIIID 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...
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 snRNP are sensitive to the transcriptional activity of the cell might indicate that the U1-TAF15 snRNP have a Pol II transcription-dependent function. By sequestering U1 snRNA into the U1-TAF15 snRNP, 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 500μg of nuclear extract were immunoprecipitated with 50μl of protein G-Sepharose (Amersham Pharmacia Biotech, Uppsala, Sweden) and approximately 5–10μg 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′-32P]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
linearized and used as templates for in vitro transcription with the T7 RNA polymerase (Promega, France) in the presence of \( \alpha\)\(^{32}\)P\(\text{CTP (30 Ci/mmol).} \)

**Immunofluorescence and in situ hybridization.** Fixation, permeabilization, immunostaining, in situ hybridization of HeLa cells, synthesis and chemical conjugation of amino-modified oligo-nucleotides 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).

**ACKNOWLEDGEMENTS**

We thank S. Muller and R. Lührmann for antibodies, and C. Bourgeois and J. Stévenin for advice. L.J. was supported by a fellowship from the Ministère de l’Éducation Nationale, de la Recherche et de la Technologie (MERT) and by Association pour la Recherche sur le Cancer (ARC). This study was financially supported by Centre National de la Recherche Scientifique (CNRS), Institut National de la Santé et de la Recherche Médicale (INSMR), Association for International Cancer Research (AICR) (03-084 and 09-025B) to L.T. and by Université PaulSabatier, Ligue Nationale Contre le Cancer, Fondation pour la Recherche Médicale en France (FRM) and Agence Nationale de la recherche (ANR) (05-BLAN-0318-01) to T.K.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**REFERENCES**

Bertolotti A, Lutz Y, Heard DJ, Champon P, Tora L (1996) hTAFII68 a novel RNA/ssDNA-binding protein with homology to the pro-oncoproteins TLS/FUS and EWS is associated with both TFIID and RNA polymerase II. **Mol
cell Biol** 16: 5022–5031

Bertolotti A, Bell B, Tora L (1999) The N-terminal domain of human TAFII68 displays transactivation and oncogenic properties. **Oncogene** 18: 8000–8010

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

Chari A, Golas MM, Klingenhager M, Neuenkirchen N, Sander B, Englbrecht C, Sickmann A, Stark H, Fischer U (2008) An assembly chaperone collaborates with the SMN complex to generate spliceosomal snRNPs. **Cell** 135: 497–509

Damgaard CK, Kahns S, Lykke-Andersen S, Nielsen AL, Jensen TH, Kjems J (2008) A S’ splice site enhances the recruitment of basal transcription initiation factors in vivo. **Mol
cell Biol** 29: 271–278

Gunderson SJ, Polycarpou-Schwarz M, Mattaj IW (1998) U1 snRNP inhibits pre-mRNA polyadenylation through a direct interaction between U1 70K and poly(A) polymerase. **Mol
cell Biol** 1: 255–264

Hicks MJ, Yang CR, Kofajich MV, Hertel KJ (2006) Linking splicing to Pol II transcription stabilizes pre-mRNAs and influences splicing patterns. **PloS Biol** 4: e147

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

Jobert L, Argentini M, Tora L (2009) PRMT1 mediated methylation of TAF15 is required for its positive gene regulatory function. **Exp Cell Res**, [doi:10.1016/j.yexcr.2008.12.008]

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

Kwek KY, Murphy S, Burger A, Thomas B, O’Gorman W, Kimura H, Proudfoot NJ, Akoulitchev A (2002) U1 snRNA associates with TFIIH and regulates transcriptional initiation. **Nat Struct Biol** 9: 800–805

Kyriakopoulou C, Larsson P, Liu L, Schuster J, Soderbom F, Kirekbo LM, Virtanen A (2006) U1-like snRNAs lacking complementarity to canonical S’ splice sites. **RNA** 12: 1603–1611

Law WJ, Cann KL, Hicks GG (2006) TLS, EWS and TAF15: a model for transcriptional integration of gene expression. **Brief Funct Genomic Proteomic** 5: 8–14

Stanek D, Prilada-Halicova J, Novotny I, Huranova M, Blazikova M, Wen X, Sapra AK, Neugebauer KM (2008) Spliceosomal small nuclear ribonucleoprotein particles repeatedly cycle through Cajal bodies. **Mol Biol Rep** 35: 2534–2543

Surowy C (1989) Direct, sequence-specific binding of the human U1-70K ribonucleoprotein antigen protein to loop I of U1 small nuclear RNA. **Mol Cell Biol** 9: 4179–4186

Wang X, Arai S, Song X, Reichart D, Du K, Pascual G, Tempest P, Rosenfeld MG, Glass CK, Kurokawa R (2008) Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription. **Nature** 454: 126–130

Will CL, Lührmann R (2006) Spliceosome Structure and Function. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press

**EMBO reports** is published by Nature Publishing Group on behalf of European Molecular Biology Organization. This article is licensed under a Creative Commons Attribution-NonCommercial-Share Alike 3.0 License. [http://creativecommons.org/licenses/by-nc-sa/3.0/]

---

**Human U1 snRNA forms a new snRNP with TAF15**

L. Jobert et al.