Volume 267, number 1, 121–125 FEBS 08569 July 1990

Presence of a differentially expressed U3A RNA variant in mouse

Structure and evolutive implications

S. Mazan, L.H. Qu, J. Sri-Widada*, M. Nicoloso and J.P. Bachellerie

Centre de Recherche de Biochimie et Génétique Cellulaires du CNRS, 118 route de Narbonne, 31062 Toulouse Cédex, France

Received 9 April 1990; revised version received 7 May 1990

A U3 RNA variant has been identified in mouse, the abundance of which relative to the previously characterized major form (U3B) appears to vary to a large extent depending upon the cell origin. Its partial sequence analysis shows that it is clearly related to the U3A form previously described in rat. Sequence comparisons suggest that the separation of the two forms of U3 genes now found in rat and mouse represent a relatively ancient event in rodent evolution. While mouse U3B RNA is encoded by four clustered genes, the U3A variant is encoded by a unique gene. Both mouse U3 RNAs differ substantially in primary structure (more than 10% divergence) Although rodent U3 RNAs exhibit a largely similar secondary structure, a specific difference between the A and B form can nevertheless be observed.

U3 RNA; Sequence; Secondary structure; Evolution; Rodent, rRNA processing

1. INTRODUCTION

For several snRNA species involved in pre-mRNA splicing, multiple variant forms may be encoded in the same genome and be expressed differently during development [1,2]. Changes in the relative amounts of variant forms might be involved in the control of the differential splicing of pre-mRNA at different stages of differentiation [3].

U3 small nucleolar RNA functions in pre-rRNA processing [4] through molecular mechanisms which remain presently unknown [5]. So far, U3 RNA variants have been reported in only two eucaryotes, yeast *S. cerevisiae* and rat. In *S. cerevisiae*, the two variant forms, snR17A and snR17B (which exhibit an 8% sequence divergence), are each encoded by a unique gene [6,7]. While the deletion of both genes is lethal, deleting one or other of the genes has no obvious phenotypic effect [6]. However, snR17B exhibits dosage compensation in the absence of snR17A (which is normally 5–10-fold more abundant than snR17B). In rat, three distinct U3 species have been identified [8] but no information has been reported regarding their possible differential expression. While the U3C variant differs from the most abundant U3B form by a single nucleotide change, the U3A species exhibits much more extensive differences (13% divergence). The rat haploid genome contains about 5–10 copies of U3 genes but the precise copy number for each variant is not known. A single gene copy has been isolated so far [9] for rat U3A and U3B and sequences immediately flanking their RNA coding regions have been shown to diverge extensively, in line with the possibility of different mechanisms of transcriptional control. Mouse represents an interesting system for further studying the role of U3 RNA, due to the recent development of an in vitro assay for one of the rRNA processing reactions [4] and also because it represents one of the 3 eucaryotes for which a complete pre-rRNA primary structure has been reported [10]. Moreover, the 4 functional genes which encode the major U3B RNA form in mouse have been recently characterized ([11] and Mazan, S. and Bachellerie, J.P., submitted). In the present study, we have identified a mouse U3 RNA variant which can be differentially expressed in various mouse cell types (as compared to the more abundant U3B form). The structural analysis of this mouse variant which is encoded by a unique gene reveals that it is clearly related to the U3A form identified in rat. It also shows that the separation of U3B and U3A genes represents an ancient event in the evolution of rodents.

Correspondence address: J.P. Bachellerie, Centre de Recherche de Biochimie et Génétique Cellulaires du CNRS, 118 route de Narbonne, 31062 Toulouse Cédex, France

* *Present address:* Centre de Recherche de Biochimie Macromoléculaire du CNRS, BP 5051, route de Mende, 34033 Montpellier, France

2. MATERIALS AND METHODS

About 2.5 mg of nuclear RNA extracted from mouse liver nuclei by the LiCl/urea procedure [12] were first separated onto a 2-mm-thick 5% acrylamide/7 M urea gel [13], using four 2.5 cm wide lanes. Electrophoresis conditions were chosen so as to achieve approximately a 10 cm migration for U3 RNA (500 V, 3 h 30 min, for a 27-cm-long gel). After electrophoresis, in order to reveal the position of the

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

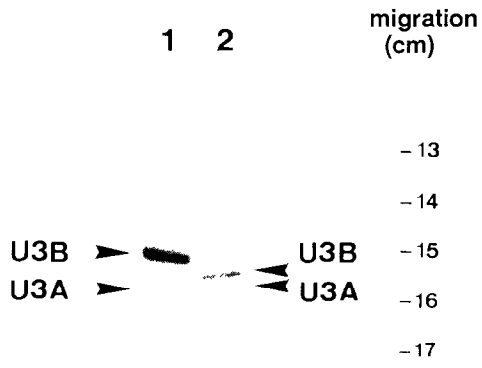

Fig. 1. Separation of the mouse U3 RNA variant. Four μg aliquots of total cellular RNA from mouse 3T3 cells (lane 1) or from rat liver (lane 2) were separated on an 8% acrylamide-0.4% bisacrylamide-7 M urea gel in 50 mM Tris-boric acid (pH 8.3), 1 mM EDTA. After electrophoresis, RNA was electrotransferred onto a nylon membrane and U3 RNAs were revealed by hybridization with an α-^{32}P-labeled, nick-translated DNA probe corresponding to the entire RNA coding region of a mouse U3B gene [11].

unique U3 RNA band, a presoaked nylon membrane (Hybond N, Amersham) was put onto the gel for 2 min and submitted to Northern blot hybridization [17] with a ^{32}P-labeled U3 RNA probe (as in the experiment of Fig. 1) while the gel was stored at −80°C. Total U3 RNA was recovered from 1 cm slices of the frozen gel around the hybridization signal according to standard procedures [13]. The two variant forms were then separated from this total U3 RNA fraction by another electrophoresis on a more concentrated acrylamide gel, under the conditions of the experiment in Fig. 1. Sequence determinations [14–16] were performed as indicated in Fig. 1.

Genomic DNA was isolated from BALB/c mouse liver by proteinase K/SDS treatment followed by phenol extraction, digested by restriction enzymes, fractionated on 1% agarose gels and transferred to a nylon membrane (Hybond N, Amersham) essentially according to Maniatis et al. [17]. Hybridization of Southern blots with the labeled synthetic oligodeoxynucleotide was performed at 55°C in 5 × SSC, 1 × Denhardt's solution, 10 mM sodium phosphate buffer (pH 7.0), 0.5% SDS, in the presence of 20% formamide, 125 μg/ml yeast tRNA and 125 μg/ml *E. coli* DNA. The hybridization probe had been 5′ end-labeled with [γ-^{32}P]ATP (5000 Ci/mmol) using T4 polynucleotide kinase (spec. act. of the labeled probe, 2.5 × 10^7 cpm/μg). Hybridization of the membrane was carried out overnight in the presence of 2.5 × 10^5 cpm/ml of labeled probe. After hybridization, the membrane was washed several times in 0.1 × SSC, 0.1% SDS (twice at 20°C for 30 min, then 3 times at 37°C).

3. RESULTS AND DISCUSSION

When total RNA extracted from mouse cells is separated by electrophoresis on urea-acrylamide gels (acrylamide concentrations ranging from 6 to 12%) and submitted to Northern blot hybridization with a mouse U3 DNA probe [11], the hybridization signal can be resolved into two labeled bands provided the migration is long enough. A minor, faster migrating, band was detected in all the mouse cell types or tissues tested so far. However, its abundance relative to the overall U3 RNA content exhibited large and reproducible varia-

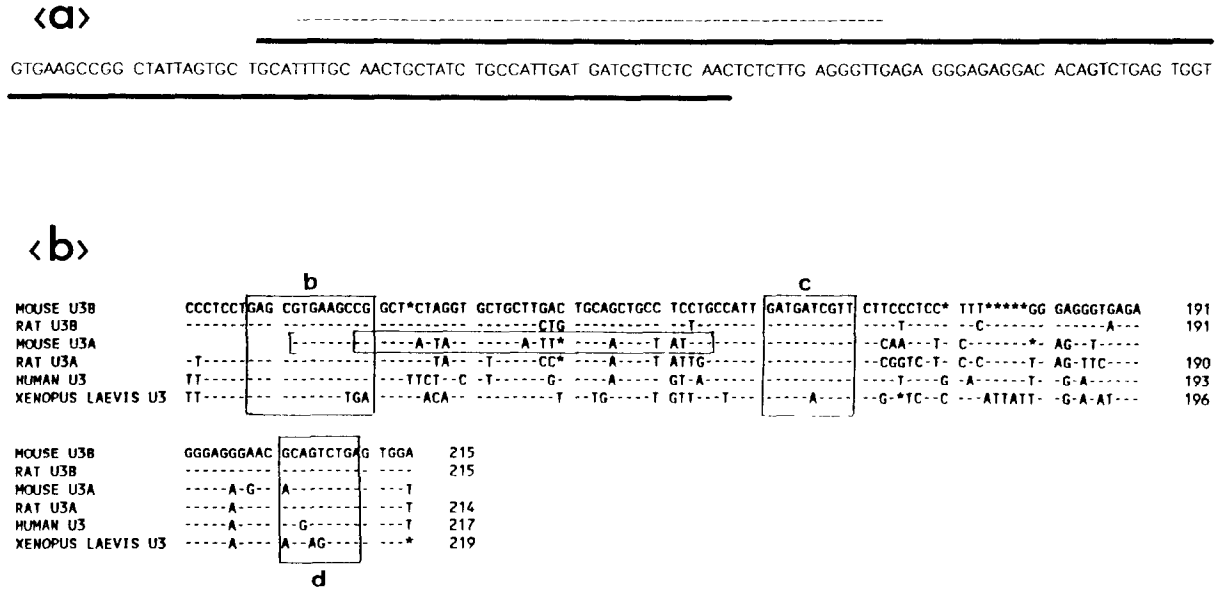

Fig. 2. Sequence of the mouse U3 RNA variant and comparison with other vertebrates. (a) The sequence shown corresponds to the 3′ terminal portion of the molecule (104 nucleotides). It was derived by the combined utilization of three techniques, with the extent of sequence determined in each case denoted by lines: (i) chemical RNA sequencing [14] after 3′ end-labeling by [^{32}P]pCp: thick overline; (ii) DNA sequencing [15] after PCR amplification [18] of a cDNA obtained by reverse transcriptase extension [16] of a synthetic oligonucleotide primer: dotted overline; (iii) direct RNA sequencing by primer extension [16]: thick underline. (b) Comparison with the other vertebrate U3 sequences: the mouse U3B sequence [11] serves as a reference (hyphens denote identities and a star a missing nucleotide). The 3 boxed portions (termed b–d) of the alignment delineate regions of highly conserved sequences in eucaryotes [11]. The motif boxed in the mouse U3A sequence has been used (as a synthetic oligodeoxynucleotide probe) to identify the corresponding sequence in mouse genomic DNA (see Fig. 4). The human and *Xenopus* sequences are taken from [19] and [20], respectively.

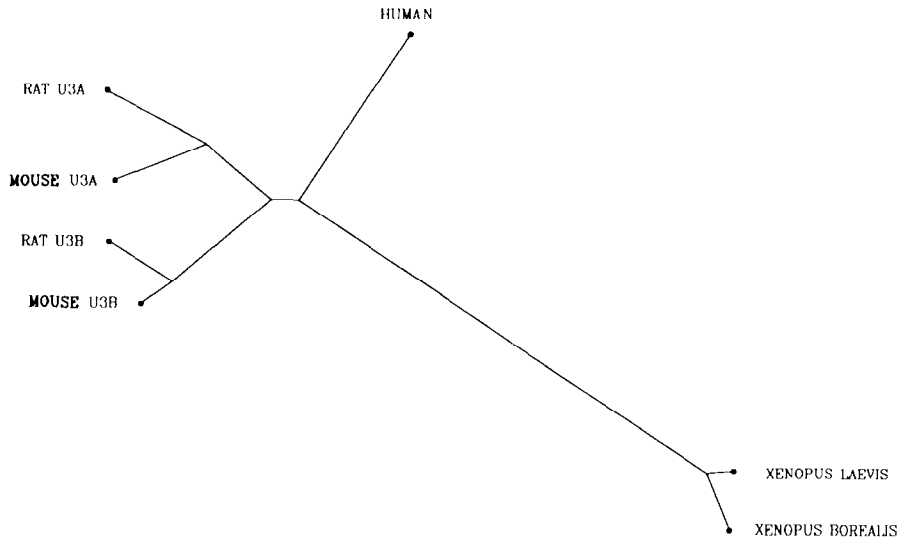

Fig. 3. Sequence relationships among vertebrate U3 RNA sequences. This unrooted tree was deduced from the comparison of the sequences aligned in Fig. 2. Distance matrixes were obtained for the portion of the sequence determined for the mouse variant and the tree derived by using the Fitch program [21] of Felsenstein's Phylip computer package.

tions according to the origin of the cells. A densitometric analysis of series of Northern blot autoradiographs (in the conditions used for the Fig. 1 experiment) shows that the relative amount of the faster migrating band is very low in brain and liver (4 and 6%, respectively), tissues for which the cell turnover is very slow. However, it is markedly increased in bone marrow (13%) which contains stem cells capable of further differentiation, and in exponentially growing 3T3 cells (in which it represents 21% of total U3 RNA).

The partial sequence analysis of this RNA fraction (Fig. 2) shows that it does not represent a degradation product of the major form but corresponds to a distinct gene product (24 nucleotide differences over the analyzed portion) which is definitely more related to rat U3A RNA than to mouse U3B RNA (Fig. 3). Several sequence ambiguities have not been resolved so far over the 5′ half of the molecule (sequence not shown): the sequence analysis of this region by primer extension methods is hampered by the particular susceptibility to endonucleolytic cleavage during reverse transcription of the phosphodiester bond between position +92 and +93 (using mouse U3B RNA sequence [11] coordinates). However, since this portion of the sequence is strongly conserved among the A and B forms of rat (only 4 differences, as compared to 28 differences over the 3′ half), it is likely to be poorly informative (as compared to the 3′ half), for evaluating the genetic relationships among the various vertebrate U3 sequences available so far. The two amphibian sequences [20] provide an outgroup for identifying the node corresponding to the primates/rodents divergence (Fig. 3), thus suggesting that the diversification of the U3A and U3B variant genes is posterior to the mammalian radiation, but represents a rather ancient event in rodent evolution.

The U3 RNA secondary structure has been shown to be largely conserved among vertebrates [9,11,20], with an accumulation of compensatory base changes over its four major stem regions. Mouse U3A RNA sequence data are in general agreement with this observation, at least for the portion determined so far. However, the availability of a second specimen of rodent U3A form reveals a distinctive feature of its folding pattern as compared to the rodent U3B forms. As shown in Figs 2 and 4, helix III represents a major area of sequence

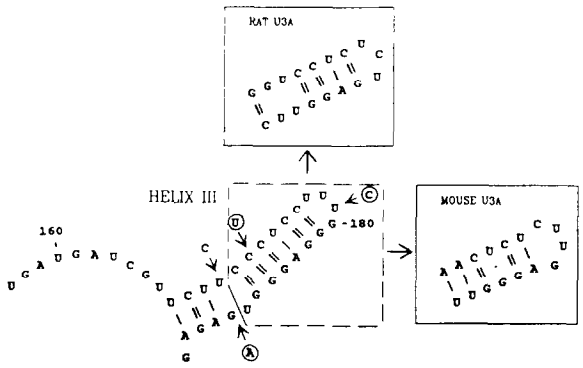

Fig. 4. A secondary structure feature specific to U3A RNA in rodents. Region (+157, +192) of the folded mouse U3B RNA sequence [11] is shown. The portion boxed with a dotted line has accumulated nucleotide changes between rodent B and A forms (with the corresponding portion for mouse and rat U3A RNAs depicted in insets). Substitutions in rat U3B are indicated (circled nucleotides). Outside the boxed portion, the single nucleotide difference in rodent U3A RNAs relative to mouse U3B is also shown.

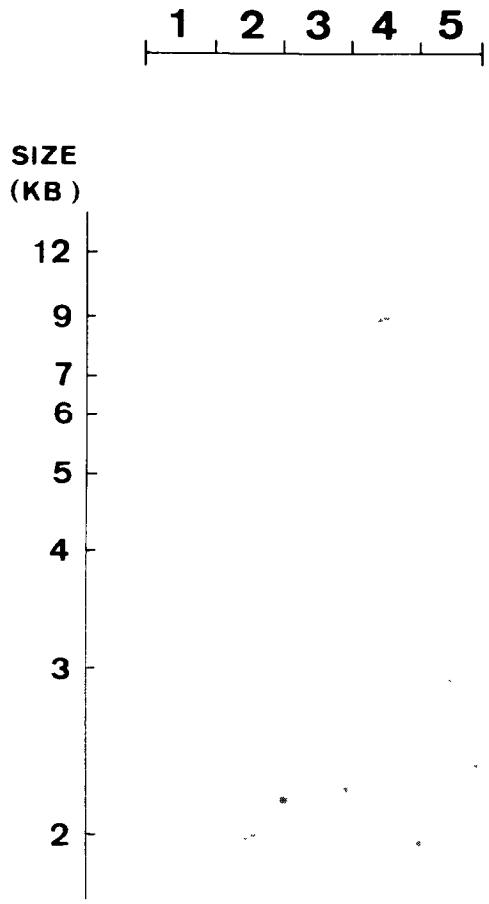

Fig. 5. Southern blot analysis of mouse DNA with a U3A RNA specific probe. Genomic DNAs digested by *Bam*HI (lane 1), *Eco*RI (2), *Hin*dIII (3), *Pvu*II (4) or *Sac*I (5) have been electrophoresed on a 1% agarose gel, transferred onto a nylon membrane and hybridized with a 5′ ^{32}P-labeled synthetic oligonucleotide (boxed in Fig 2) recognizing selectively the U3A variant form.

variation between the A and B forms of rodents. Several nucleotide changes are also observed in this area for U3A between rat and mouse which nevertheless preserve the regularity of the stem. By contrast, a bulged nucleotide is found, while in the B-form version of this stem, which is maintained at the same position in both rodents, compensatory base changes between mouse and rat are detected over adjacent base-pairings. This subtle difference between the A and B structures could be biologically significant, taking into account that a bulge nucleotide may represent a key element for protein-RNA recognition [22]. It could be related to some unknown difference in the structural organization of the corresponding U3 snRNPs, the cellular substrate for U3RNA function [4,23]. In mouse, U3B RNA is encoded by 4 functional genes which are clustered in a single chromosomal locus ([11] and Mazan, S. and Bachellerie, J.P., submitted). A synthetic oligonucleotide (35-mer) probe was selected in a region of the mouse U3 RNA sequence where a large number of nucleotide differences between the A and B forms have accumulated (Fig. 2, bottom) in order to recognize selectively U3A genes in a Southern blot hybridization of mouse genomic DNA. As shown in Fig. 5, a unique radioactive band is detected for each of the 5 different digests analyzed (using enzymes with a 6-nucleotide recognition sequence) indicating that mouse U3A RNA is encoded by a unique gene. As expected, a strong signal is also observed at the same position in each lane (result not shown) when the same blots are probed with a labeled DNA fragment corresponding to the entire mouse U3B coding region [11]; this signal is distinct from the ones corresponding to the previously identified functional U3B genes and to non-functional retrogenes [24]. The cloning of this unique mouse U3A gene (now in progress) should help to assess the mechanisms involved in the differential expression of both RNA forms (particularly at the transcription level) according to the growth rate or development stage. The characterization of two markedly different forms of U3 RNA which have been preserved throughout a long period of rodent evolution should also provide novel clues regarding the detailed molecular mechanism of action of U3 in pre-rRNA processing.

REFERENCES

[1] Forbes, D.J., Kirschner, M.W., Caput, D., Dahlberg, J.E. and Lund, E. (1984) Cell 38, 681–689.
[2] Lund, E., Kahan, B. and Dahlberg, J.E. (1985) Science 229, 1271–1274.
[3] Dahlberg, J.E. and Lund, E. (1988) in: Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles (Birnstiel, M.L. ed.) pp. 38–70, Springer, Berlin.
[4] Kass, S., Tyc, K., Steitz, J.A. and Sollner-Webb, B. (1990) Cell (in press).
[5] Steitz, J.A., Black, D.L., Gerke, V., Parker, K.A., Kramer, A., Frendewey, D. and Keller, W. (1988) in: Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles (Birnstiel, M.L. ed.) pp. 115–154, Springer, Berlin.
[6] Hughes, J.M.X., Konings, D.A.M. and Cesareni, G. (1987) EMBO J. 6, 2145–2155.
[7] Myslinski, E., Segault, V. and Branlant, C. (1990) Science (in press).
[8] Reddy, R. and Busch, H. (1988) in: Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles (Birnstiel, M.L. ed.) pp. 1–37, Springer, Berlin.
[9] Stroke, I.L. and Weiner, A.M. (1985) J. Mol. Biol. 184, 183–193.
[10] Bourbon, H., Michot, B., Hassouna, N., Feliu, J. and Bachellerie, J.P. (1988) DNA 7, 181–191.
[11] Mazan, S. and Bachellerie, J.P. (1988) J. Biol. Chem. 263, 19461–19467.
[12] Autfray, C. and Rougeon, F. (1980) Eur. J. Biochem. 107, 303–314.
[13] Maxam, A.M. and Gilbert, W. (1980) Methods Enzymol. 65, 499–560.
[14] Peattie, D.A. (1979) Proc. Natl. Acad. Sci. USA 76, 1760–1764.
[15] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.

[16] Qu, L.H., Michot, B. and Bachellerie, J.P. (1983) Nucleic Acids Res. 11, 5903–5920.
[17] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[18] Mullis, K.B. and Faloona, F.A. (1987) Methods Enzymol. 155, 335–350.
[19] Suh, D., Busch, H. and Reddy, R. (1986) Biochem. Biophys. Res. Commun. 137, 1133–1140.
[20] Jeppesen, C., Stebbins-Boaz, B. and Gerbi, S.A. (1988) Nucleic Acids Res. 16, 2127–2148.
[21] Fitch, W.M. and Margoliash, E. (1967) Science 155, 279–284.
[22] Peattie, D.A., Douthwaite, S., Garrett, R.A. and Noller, H.F. (1981) Proc. Natl. Acad. Sci. USA 78, 7331–7335.
[23] Parker, K.A. and Steitz, J.A. (1987) Mol. Cell. Biol. 7, 2899–2913.
[24] Mazan, S., Michot, B. and Bachellerie, J.P. (1989) Eur. J. Biochem. 181, 599–605.