Volume 16 Number 13 1988 Nucleic Acids Research

Heterogeneity of human U1 snRNAs

Elsebet Lund

Department of Physiological Chemistry, University of Wisconsin-Madison, Madison, WI 53706, USA

Received March 30, 1988; Revised and Accepted June 6, 1988

ABSTRACT

I demonstrate that the U1 snRNAs of human cells are heterogeneous in sequence. Polyacrylamide gel and RNase T1 fingerprint analyses of U1 RNAs isolated from a variety of human cultured cells, including HeLa, 293, K562 and NT2/D1, show that minor variants of the human U1 RNA (hU1a) comprise between 5% and 15% of the total U1 RNAs in these established cell lines. The patterns of variants are cell line specific, suggesting that expression of these minor species of hU1a RNAs reflect polymorphisms of the hU1a true genes rather than existence of an additional class of human embryonic U1 genes. Also, the hU1a variants described here are not the products of previously identified human U1 Class I pseudogenes.

INTRODUCTION

In human cells, the most abundant of the small nuclear RNAs, U1 RNA (1), has been shown by direct RNA sequence analyses to consist of a single species, referred to as U1a (2). Human U1a RNA (hU1a) is encoded by a large cluster of about 30 genes (3,4), the RNU1 locus, which has been mapped to 1p36 on the short arm of chromosome 1 (5,6). In addition to the true U1 genes, the human genome contains a large number of U1 pseudogenes which apparently are not expressed in human cultured cells (7). This includes a cluster of Class 1 U1 pseudogenes (8) (located at 1q12-1q22 on the long arm of chromosome 1 [9]) which share extensive sequence homology with both coding and 5' flanking regions of the true human U1 genes (8,10).

In mouse cells, multiple variants of U1 RNAs have been identified, which on the basis of sequence can be assigned to one of two major classes, the mU1a and mU1b RNAs (11-13). Besides their structural differences, these two classes of mouse U1 RNAs are distinguished by the control of their expression

during development: mU1a RNAs ("adult") are synthesized constitutively in all cell types, but mU1b RNAs ("embryonic") accumulate only in fetal tissues and in adult tissues containing stem- or germ-line cells capable of further differentiation (12). Moreover, genes for mU1a and mU1b RNAs are not closely linked in the mouse genome, which contains at least three distinct loci of U1 RNA true genes (14-16).

Murine cells of established lines synthesize varying amounts of mU1b RNAs, presumably reflecting the developmental lineage and, hence, the level of mU1b RNA of the original cell isolate (12). Thus, mouse embryonal carcinoma (EC) and mouse erythroleukemia (MEL) cells contain 50-60% mU1b RNAs, whereas mouse fibroblasts (e.g. C127 and 3T3 cells) produce little, if any, mU1b RNAs (11-13,17).

To determine if human cells also have the capacity to make U1b-type RNAs I have analyzed the U1 RNAs of a variety of established human cell lines. I demonstrate here that, in addition to the major species of human U1 RNA several minor sequence variants of hU1a RNA are made in all cell lines tested, including HeLa, 293, K562 and NT2/D1. However, the populations of minor hU1a RNAs vary between cell lines, and none of the variant human U1 RNAs resembles the mouse embryonic U1b RNAs in sequence. I conclude, therefore, that the expression of hU1a variants most likely results from minor polymorphisms in the complement of hU1a true genes, rather than from an additional class of human (hU1b-like) genes.

MATERIALS AND METHODS

Cell lines.

NT2/D1 cells, a clonal derivative of human teratocarcinoma cell line Tera-2 (18), were provided by Dr. P. Andrews (The Wistar Institute, Philadelphia) and were maintained at high density in DME (Dulbeccos Modified Eagle) medium supplemented with 10% FCS (fetal calf serum). K562 cells, a human erythroleukemia cell line expressing embryonic globin (19), were obtained from J. Ross (University of Wisconsin-Madison), and were kept in suspension culture in RMPI 1640 medium containing 10% FCS. 293 cells, a line of adenovirus transformed human cells

(20), HeLa cells, derived from a human cervical carcinoma (21), and CV-1 cells, an established line of African green monkey kidney cells (22), were grown in DME medium supplemented with 10% FCS. Daudi and 884 cells, Epstein-Barr Virus (EBV) transformed human lymphoblastoid cells were provided by R. DeMars (University of Wisconsin-Madison), and 356 cells, a strain of diploid human foreskin fibroblasts were supplied by Brenda Kahan (University of Wisconsin-Madison). Mouse 1848 and E102 cells, lines of Abelson virus-transformed pre-B cells, were obtained from R. Risser (University of Wisconsin-Madison). Mouse C127/189a cells, a line of hU1/BPV-transformed C127 cells, was generated in this laboratory (E.T. Schenborn, E.J. Bostock and E.L., unpublished results).

Preparation of RNA.

Total RNAs were isolated from exponentially growing cultures ($2-5 \times 10^6$ cells) by the urea-lysis method of Ross (23) (omitting the CsCl-centrifugation step), precipitated by ethanol and resuspended in 50-100 µl TE-buffer (10 mM Tris-Hcl, pH 7.6, 1 mM EDTA). To obtain radioactively labeled U1 RNAs, cells at 70% confluency were labeled for 18-20 hours with 1 mCi of ^{32}P-ortho-phosphate per 5 ml of phosphate-free DME medium containing 10% dialysed FCS and hybrid-selected U1 RNAs were isolated as previously described (5).

Antibody precipitation. ^{32}P-labeled snRNPs were isolated from clarified cell-sonicates by incubation with RNP or Sm human SLE antisera as described (11).

Analyses of U1 RNAs.

^{32}P-labeled U1 RNAs were purified from the total nucleic acids by electrophoresis at room temperature for 18 hours at 12.5 V/cm in 12% (30:0.8) polyacrylamide, 7 M urea, 1 x TEB (90 mM Tris-borate, pH 8.3, 2 mM EDTA) gels; under these conditions of partial denaturation hU1a RNAs migrates ahead of 5.8S rRNA. Further fractionation of the total, hybrid-selected or antibody-precipitated U1 RNAs were by electrophoresis in the cold room for 20 hours at 22.5 V/cm in nondenaturing 15% (19:1) polyacrylamide, 1/2 x TEB gels (5,12). The U1 RNAs in individual bands were eluted from the gel and further characterized by two-dimensional RNAse T1 fingerprinting (24) according to Barrell

(25), using Homomix C and polyethyleneimine (PEI) thin-layer plates for the 2nd dimension.

Northern blot hybridization.

Total RNAs were fractionated in 15% native gels as above and the U1 RNAs were transferred electrophoretically to Gene Screen Plus (NEN) or Zetabind (Cuno Inc.) nylon membranes and hybridized with an SP6 anti-U1, ^{32}P-labeled RNA probe as previously described (12).

RESULTS

The U1 RNAs of human EC cells are heterogeneous in sequence.

To determine whether embryonic U1 RNAs similar to the mouse mU1b RNAs existed in human cells I first examined the U1 RNAs of human embryonal carcinoma (EC) cells, line NT2/D1 (18). In the mouse system, EC cells, which correspond to very early stages of embryonic development (26), contain high levels of mU1b RNAs (12). When total ^{32}P-labeled U1 RNAs were isolated from the comparable human EC cells, and analyzed by electrophoresis in a partially denaturing polyacrylamide gel (Figure 1A), only a single band of U1 RNA was observed (lane 1) which comigrated with mouse mU1a (lane 2) or HeLa hU1a RNAs (data not shown). This is in contrast to the multiple bands of mU1a and mU1b RNAs seen in some samples of mouse U1 RNAs (lane 3).

To resolve possible variant RNAs present in the single band of NT2/D1 hU1a RNAs, the same preparation of hybrid-selected RNA was analyzed in a nondenaturing gel (Figure 1B). Several bands of U1 RNAs were observed (lane 1); together, the minor species accounted for more than 10% of the total NT2/D1 U1 RNAs (Table 1). The same pattern of bands was obtained when the NT2/D1 U1 RNAs were first isolated by precipitation with RNP- or Sm-antisera (data not shown); this indicated that the RNAs of the minor bands were incorporated into U1 snRNPs (11) and thus probably were functional U1 RNAs (27).

The U1 RNAs of the major and minor bands were characterized further by two-dimensional RNase T1-fingerprinting (24,25) as illustrated in Figure 2. At least two of these bands appeared to contain a mixture of variant and wildtype hU1a RNAs (compare the fingerprints of NT2/D1 a-1, a-2 and a-3 U1 RNAs and see legend

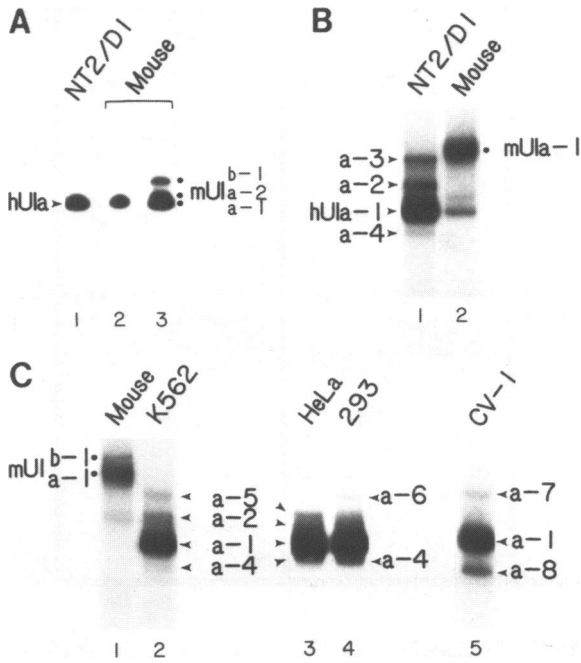

Figure 1. Heterogeneity of human U1 RNAs.
(A) Hybrid-selected, ^{32}P-labeled U1 RNAs of human NT2/D1 (lane 1) and mouse C127/189a (lane 2) and E102 (lane 3) cells were fractionated in a partially denaturing (12%, 7 M Urea) gel. These two lines of mouse cells differ in their expression of mU1a and mU1b RNAs (12).
(B) U1 RNAs of human NT2/D1 (lane 1) and mouse C127/189a (lane 2) cells were separated in a non-denaturing (15% gel). Mouse C127/189a cells express both mU1a-1 and hU1a-1 RNAs.
(C) U1 RNAs of human K562, HeLa and 293 (lanes 2-4), mouse 1848 (lane 1) and African green monkey CV-1 cells (lane 5) were fractionated as in (B). Autoradiograms are shown. The identity of the U1 RNAs in the various bands were determined by RNase T_1 fingerprinting (Figure 2 and data not shown).

to Figure 2), but neither of the novel U1 species was related structurally to mouse mU1b RNAs (see Figure 4). Thus, the heterogeneity of hU1 RNAs in NT2/D1 cells does not support the existence of a structurally distinct class of U1b-type RNAs in human EC cells. However, the level of methylation of nucleotide A_{70} is significantly decreased in the hU1a RNAs of NT2/D1 and K562 cells relative to other human cell types (e.g. compare the ratios of oligonucleotides #14 and #15 in the fingerprints of

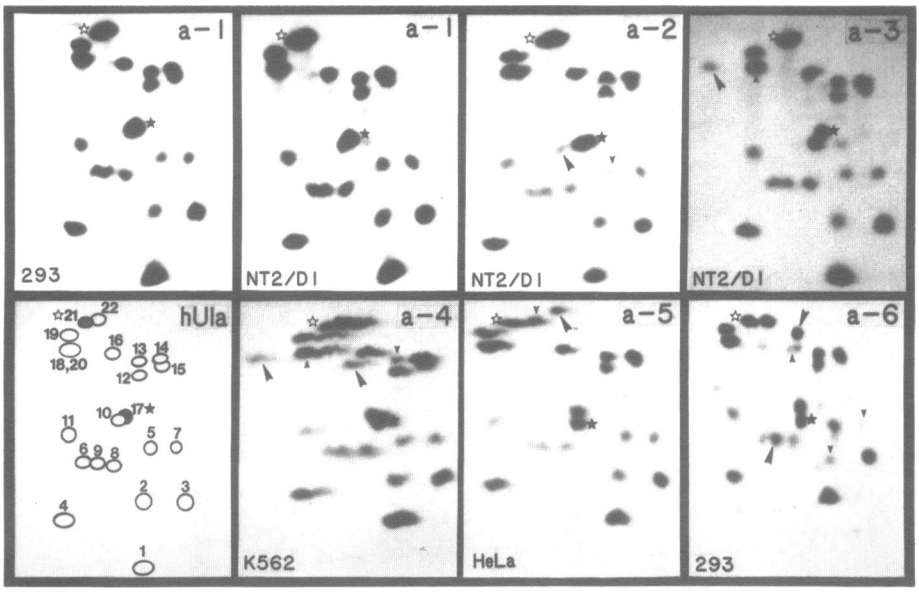

Figure 2. RNase T_1 fingerprint analyses of human U1 RNAs. RNase T_1 fingerprints of uniformly labeled hU1a RNAs purified from human cell lines 293 (a-1 and a-6), NT2/D1 (a-1, a-2 and a-3), K562 (a-4) and HeLa (a-5) as illustrated in Figure 1. Large arrowheads indicate variant-specific RNase T_1 oligonucleotides. Small arrowheads denote common hU1a-1 oligonucleotides which are not derived from the variant RNAs but which are present in sub-molar amounts in these fingerprints due to contamination of the minor U1 RNAs with hU1a-1 RNA. Contamination ranges from < 10% (a-6) to ≥ 50% (a-2, -3, -4 and -5). The schematic shows the standard numbering system of hU1a RNase T_1 oligonucleotides (30); the 5'-(#21, shaded) and 3'-(#17, filled) terminal oligonucleotides are indicated in all fingerprints by open and filled stars, respectively, because the mobilities of these oligonucleotides vary with the precise conditions of separation used. The first dimension of the fingerprints was electrophoresis at pH 3.5 (right to left), and the second dimension was homochromatography on PEI thin-layer plates (top to bottom). The a-1 fingerprints represent total hU1a RNAs of 293 or NT2/D1 cells, demonstrating the difficulty in detecting minor variant RNAs by this method alone. The molar ratio of oligonucleotides #14 and #15 is significantly >1 in all U1 RNAs of NT2/D1 and K562 cells (see also a-2, a-3 and a-4) due to incomplete ribose-methylation of residue A_{70} (data not shown); the lack of this modification causes oligonucleotide #15 (CAmCUCCGp) to comigrate with oligonucleotide #14 (ACCCCUGp).

Table 1.
Variant hU1a RNAs

U1 RNA	Sequence Change[a] in Variant RNA	RNase T1[b] Oligonucleotide	Level of Accumulation in Cultured Cells[c] NT2/D1	K562	HeLa	293	CV-1
hU1a-1	(Major Species)		85%	90%	95%	95%	85%
hU1a-2[d]	$_{35}$AAGG$_{38}$ ↓ AAUG	#5	8%	5%	<1%	–	–
hU1a-3[e]	$_{42}$UUUUCCCAG$_{50}$ ↓ UUUU($\frac{C}{2}$U)AG	#20	5%	–	–	–	–
hU1a-4[e]	Same as a-3 and $_{111}$AAACUCG$_{117}$ ↓ AAA(U$\frac{C}{2}$)G	#20 #13	3%[f]	4%	3%	2%	–
hU1a-5[e]	$_{92}$CGAUUUCCCCAAAUG$_{106}$ ↓ CCAUUUCCCCAAAUG	#22	–	3%	3%	–	–
hU1a-6[g]	$_{19}$AGAUACCAUG$_{28}$ ↓ AAAUACCAUG and $_{13}$CAG$_{15}$ → UAG	#2, #16 #7	–	–	–	2%	–
hU1a-7[g]	$_{110}$GAAACUCG$_{117}$ ↓ AAAACUCG	#13	–	–	–	–	4%
hU1a-8[g]	$_{29}$AUCACG$_{34}$ ↓ AUCAUG	#12	–	–	–	–	10%

[a]Subscript numbers indicate the position of the oligonucleotides in the nucleotide sequence of hU1a RNA (2). The lower lines show the variant-specific oligonucleotides.

[b]Numbering of the affected hU1a oligonucleotide is according to ref. 30 (cf. Figure 2).

[c]The level of accumulation was estimated from the amount of radioactivity and the relative purity of the variant RNAs in the different gel bands.

[d]This nucleotide change was confirmed by RNase A digestion of hU1a-2 RNA, showing the gain of oligonucleotide GAAUp at the expense of oligonucleotide GAAGGUp (data not shown).

[e]These nucleotide changes were confirmed by redigestion of the variant-specific oligonucleotides with RNase T_2.

[f]The presence of hU1a-4 RNA in NT2/D1 cells was inferred from the gel-mobility of the minor band (Figures 1B and 3) but was not confirmed by RNase T_1 fingerprinting analysis.

[g]These changes were deduced from the characteristic fingerprint mobilities of the novel variant-specific oligonucleotides and the concomitant decrease in the levels of specific hU1a oligonucleotides (cf. Figure 2).

hU1a-1 RNA from NT2/D1 and 293 cells and see legend to figure). The absence of this methylation is also a common feature of both mouse (12,13) and frog (28) U1b RNAs.

Variants of human U1 RNA are cell-line specific.

To determine if the expression of variant hU1a RNAs was specific to cells of the early embryonic lineages, similar electrophoretic analyses were performed on hybrid-selected, ^{32}P-labeled U1 RNAs from K562, 293 and HeLa cells. In all cases (Figure 1C), small amounts of U1 RNAs were observed that differed in electrophoretic mobility from the major species of hU1a RNA. However, the patterns of minor bands varied from cell line to cell line, and each variant RNA represented only between 2 and 5% of the total U1 RNAs in K562, 293 and HeLa cells (Table 1). Further characterization of the RNAs in individual bands confirmed the existence of several additional hU1a variants (bottom panels of Figure 2). The results of these analyses are summarized in Table 1 and Figure 4.

We conclude that the U1 RNAs of most, and perhaps all, human cell lines are slightly heterogeneous in sequence, and that these variants likely are the result of minor polymorphisms in the complement of hU1a RNA true genes, rather than of an additional class of human embryonic U1 genes.

To survey more easily the U1 RNA populations in additional lines of human cells, we used a highly sensitive Northern blot assay that allowed us to detect minor species of U1 RNA in preparations of total, unlabeled nucleic acids (12,29). Figure 3 shows an example of such analysis, which confirm the results presented above and extends them to three other lines of human cells, a strain of diploid foreskin fibroblast (356 cells, lane 1) and two lines of EBV-transformed cells, Daudi (lane 5) and 884 (lane 6).

Variant U1 RNAs are common to human and monkey cells.

African green monkey (CV-1) cells contain an RNA identical to hU1a RNA as the major species of U1 RNA as assayed by RNase T1 fingerprinting (data not shown). When CV-1 U1 RNAs were examined for heterogeneity, two additional U1 RNA variants were revealed (Figure 1C, lane 5), which together accounted for over 10% of the CV-1 U1 RNAs (Table 1). The faster migrating species of CV-1 U1

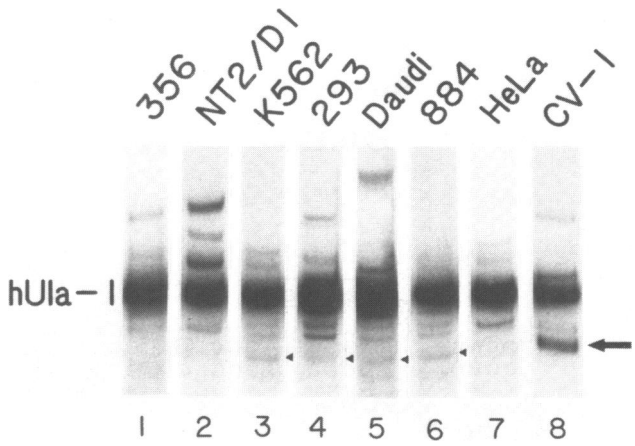

Figure 3. Northern blot analysis of human and monkey cell U1 RNAs.
Total unlabeled RNAs of the cell lines indicated were fractionated as in Figure 1B,C and assayed for U1 RNAs by northern blot hybridization using a single-stranded SP6-RNA probe. The arrow indicates the mobility of hU1a-8 RNA of CV-1 cells (cf. Fig. 1C) and small arrowheads denote minor RNAs of similar gel mobilities, which are discussed in the text.

RNAs (a-8 in Figure 1C; arrow in Figure 3) is a variant of hU1a RNA rather than a truncated form like U1* (27) (data not shown). Thus in this gel system, rapidly migrating RNAs (such as those indicated by arrowheads in Figure 3) may well be additional variants rather than U1 RNA degradation products.

DISCUSSION

I have demonstrated that minor variant hU1a RNAs are expressed in human cells. Detailed analyses of the U1 RNAs in four established lines of human cells show the presence of 5 different, novel hU1a variants (Table 1, Figure 4), indicating that the total number of variant hU1a RNAs might be quite large. The failure of earlier studies (2,7,11,30) to detect these minor species most likely reflects the low abundance of individual variant RNAs (Table 1 and Figure 2). Recently, Patton and Wieben (31) provided evidence for variation in the precursor-specific 3' termini of HeLa and K562 pre-U1 RNAs; it remains to be established, how (or if) the two types of variation in the human U1

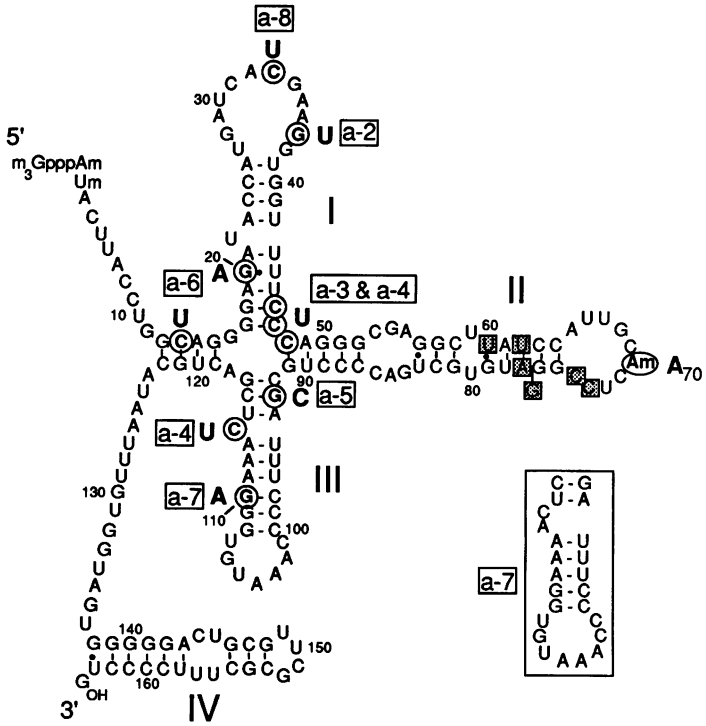

Figure 4. Secondary structure of human U1a RNAs.
The complete nucleotide sequence of human U1a RNA (2) is shown in the secondary structure proposed by Mount and Steitz (37). The nucleotide changes occurring in the variant hU1a RNAs (boxed and numbered) are indicated by circles and bold face letters; in case of "a-3 & a-4" it is unknown which of the three circled C's is changed to a U. The insert shows an altered stem-loop III structure, that might be formed in hU1a-7 RNA. The shaded squares in stem-loop II indicate the nucleotides that are altered in all mouse U1b RNAs (12,13).

genes- in the coding region and 3' flanking region, respectively- are related to each other.

In the present work, I used a nondenaturing gel system to resolve U1 RNAs of identical length, but slightly different sequence (5,12,28). We have shown previously (5) that mouse and human U1 RNAs, which differ in only 2 out of 165 nucleotides (2, 13), migrate with very different mobilities in this gel system (see also Figure 1B, C). Consistent with that observation, I was able to identify minor sequence variants of hU1a RNA that

contained either a single (e.g. hU1a-2) or just a few nucleotide changes relative to the canonical hU1a sequence (Figure 4). Although some of these changes would be expected to alter the conformation of the U1 RNA molecule significantly (e.g. hU1a-7), others were located in regions that are supposedly single-stranded (e.g. hU1a-2 and a-8). Therefore, tertiary interactions involving apparently single-stranded regions of the molecule may contribute significantly to the overall conformation of U1 RNA, at least under the conditions of gel-electrophoresis used here (see also discussion in references 13 and 32).

In most cases, changes in the mobilities of specific RNase T1 oligonucleotides (Figure 2) were sufficient to reveal the site of mutation in the variant U1 RNAs to within one or a few nucleotides (Table 1). Although the variations in hU1a RNA were localized primarily in stem-loop I and stem-loop III (Figure 4), variations in the mouse (12,13) and Xenopus U1 RNAs (28,32) are frequently located in stem-loop II and IV. The nucleotide changes described here are apparently without effect on the binding of the RNP- (70 K) and Sm- (B or D) antigens. It is not known, however, if the entire complement of proteins in snRNPs with variant U1 RNAs is normal. Likewise, it is unclear if all the variant snRNPs can participate in pre-mRNA splicing.

The genes responsible for the synthesis of the variant hU1a RNA are unknown. It is possible that the genes are variant genes belonging to the cluster of hU1a true genes on chromosome 1 (RNU1); this locus shows considerable polymorphism among individuals (3) as detected by restriction enzyme analyses of the flanking region sequences (3,4). However, the existence of previously unknown, dispersed U1 genes cannot be excluded. None of the hU1a variants described here matches the coding region sequences of the published class I U1 pseudogenes (8,10,33).

Our analyses of cell lines of early embryonic lineages (i.e. NT2/D1 and K562) reveal no consistent pattern of U1b-type RNAs in human cells. Although not an exhaustive search, these results strongly indicate that human cells lack the U1b-type genes which are expressed at high levels in embryonic or undifferentiated cells of the mouse (11,13,17). Significantly, the hU1a RNAs of NT2/D1 and K562 cells, like the U1b RNAs of

mice and frogs (12,13,28), are deficient in the ribose methylation of residue A_{70} (Figures 2 and 4). This altered methylation pattern of hU1a RNAs in cells of embryonic lineages may be functionally equivalent to the developmental control of snRNA transcription observed in mice and frogs.

The finding that the African green monkey cells express the same major species of U1a RNA as human cells (i.e. hU1a) is consistent with the observation that chromosome 1 (which carries the RNU1 locus [5,6]) is remarkably conserved among primates (34). In contrast to the primates, which span a period of about 50×10^6 years of evolution, man and mouse are separated by $80\text{-}100 \times 10^6$ years of evolution (35). These latter species differ both in the sequence of their major U1a RNA genes (i.e. hU1a vs. mU1a) and in the developmental control of expression of a second class of U1 RNA genes, the U1b genes. Among rodents, an RNA identical to mU1a RNA is also the major U1a species in rat ([1,2,13] and chinese hamster cells [data not shown]), but mU1b RNAs appear to be absent from rat tissues (1,2; E.L., unpublished results); thus, rat U1 RNAs resemble mouse U1 RNAs in sequence, but human U1 RNAs in control of expression. Since mouse and rat diverged only about $15\text{-}20 \times 10^6$ years ago (36), these observations provide evidence for the ongoing evolution of the mammalian U1 multigene families.

ACKNOWLEDGEMENTS

I thank J.E. Dahlberg for continuous support and constructive criticism of the manuscript, P. Andrews, B. Kahan, R. DeMars, J. Ross and R. Risser for supplying tissue culture cells and J.L. Mitchen for technical assistance. This work was supported by NIH grant GM30220 and NSF grant PCM-8309618 to J.E. Dahlberg and E. Lund.

REFERENCES

1. Busch, H., Reddy, R., Rothblum, L. and Choi, Y.C. (1982) Annu. Rev. Biochem. 51, 617-654.
2. Branlant, C., Krol, A., Ebel, J.P., Lazar, E., Gallinaro, H., Jacob, M., Sri-Widada, J. and Jeanteur, P. (1980) Nucl. Acids Res. 8, 4143-4154.
3. Lund, E. and Dahlberg, J.E. (1984) J. Biol. Chem. 259, 2013-2021.
4. Manser, T. and Gesteland, R.F. (1982) Cell 29, 257-264.

5. Lund, E., Bostock, C., Robertson, M., Christie, S., Mitchen, J.L. and Dahlberg, J.E. (1983) Mol. Cell. Biol. 3, 2211-2220.
6. Naylor, S.L., Zabel, B.U., Manser, T., Gesteland, R. and Sakaguchi, A.Y. (1984) Somatic Cell Mol. Genet. 10, 307-313.
7. Denison, R.A., Van Arsdell, S.W., Bernstein, L.B. and Weiner, A.M. (1981) Proc. Natl. Acad. Sci. USA 78, 810-814.
8. Denison, R.A. and Weiner, A.M. (1982) Mol. Cell. Biol. 2, 815-828.
9. Lindgren, V., Bernstein, L.B., Weiner, A.M. and Francke, U. (1985b) Mol. Cell. Biol. 5, 2172-2180.
10. Bernstein, L.B., Manser, T. and Weiner, A.M. (1985) Mol. Cell. Biol. 5, 2159-2171.
11. Lerner, M.R. and Steitz, J.A. (1979) Proc. Natl. Acad. Sci. USA 76, 5495-5499.
12. Lund, E. and Dahlberg, J.E. (1987) Genes and Devel. 1, 39-46.
13. Kato, N. and Harada, F. (1985) J. Biol. Chem. 260, 7775-7782.
14. Michael, S.K., Hilgers, J., Kozak, C., Whitney, J.B., III and Howard, E.F. (1986) Somatic Cell and Mol. Genet. 12, 215-223.
15. Lund, E. and Nesbitt, M.N. (1988) Somat. Cell Mol. Genet. 14, 143-148.
16. Blatt, C., Saxe, D., Marzluff, W.F., Lobo, S., Nesbitt, M.N. and Simon, M.I. (1988) Somat. Cell Mol. Genet. 14, 133-142.
17. Marzluff, W.F., Brown, D.T., Lobo, S and Wang, S.-S. (1983) Nucleic Acids Res. 11, 6255-6270.
18. Andrews, P.W. (1984) Develop. Biol. 103, 285-293.
19. Cioe, L., McNab, A., Hubbell, H.R., Meo, P., Curtis, P. and Rovera, G. (1981) Cancer Res. 41, 237-243.
20. Graham, F.L., Smiley, J., Russell, W.C. and Nairn, R. (1972) J. Gen. Virol. 36, 59-72.
21. Gey, G.O., Coffman, W.D. and Kubicek, M.T. (1952) Cancer Res. 12, 264-265.
22. Jensen, F.C., Girardi, A.J., Gilden, R.V. and Koprowski, H. (1964) Proc. Natl. Acad. Sci. USA 52, 53-59.
23. Ross, J. (1976) J. Mol. Biol. 106, 403-420.
24. Sanger, F., Brownlee, G.G. and Barrell, B.G. (1965) J. Mol. Biol. 13, 373-398.
25. Barrell, B.G. (1971) In Cantoni, G.L. and Davis, D.R. (ed.), Procedures in nucleic acid research, Vol. 2 Harper and Row, Publishers, New York, pp. 751-779.
26. Martin, G.R. (1980) Science 209, 768-776.
27. Lerner, M.R., Boyle, J.A., Mount, S.M., Wolin, S.L. and Steitz, J.A. (1980) Nature 283, 220-224.
28. Forbes, D.J., Kirschner, M.W., Caput, D., Dahlberg, J.E. and Lund, E. (1984) Cell 38, 681-689.
29. Lund, E. and Dahlberg, J.E. (1987) Genes and Devel. 1, 39-46.
30. Nohga, K., Reddy, R. and Busch, H. (1981) Cancer Res. 41, 2215-2220.
31. Patton, J.G. and Wieben, E.D. (1987) J. Cell Biol. 104, 175-182.
32. Krol, A., Lund, E. and Dahlberg, J.E. (1985) EMBO J. 4, 1529-1535.

33. Monstein, H.-J., Hammarström, K., Westin, G., Zabielski, J., Philipson, L. and Pettersson, U. (1983) J. Mol. Biol. 167, 245-257.
34. Finaz, C., Long, N.V., Cochet, C., Frezal, J. and deCrouchy, J. (1977) Cytogenet. Cell Genet. 18, 160-164.
35. Lalley, P.A., Francke, V. and Minna, J.D. (1978) Proc. Natl. Acad. Sci. USA 75, 2382-2386.
36. Hendriks, W., Leunissen, J., Nevo, L., Bloemendal, H. and deJong, W.W. (1987) Proc. Natl. Acad. Sci. USA 84, 5320-5324.
37. Mount, S.M. and Steitz, J.A. (1981) Nucl. Acids. Res. 9, 6351.