
Transcriptional inactivity of Alu repeats in HeLa cells

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ABSTRACT

The in vivo transcription of human Alu family members has been investigated by a sensitive primer extension method. The selected primers represent various regions of the Alu family consensus sequence, thus assaying the transcriptional activity of the entire family rather than the activity of an individual member sequence. Using this method, a very small number of RNA molecules per HeLa cell is found to have a distribution of 5' ends centered on the in vitro Alu transcription start site. The distribution of these 5' ends suggests that they are more likely the result of hnRNA degradation rather than transcription start sites. Therefore, despite their great numerical abundance, Alu family members are transcriptionally silent in HeLa cells.

INTRODUCTION

The 500,000 members of the human Alu family of repeated sequences are broadly distributed throughout the genome and share a 300 bp consensus sequence [1]. Individual Alu family repeats are usually surrounded by short direct repeats and terminate in a poly A rich 3' end [1]. These features suggest Alu family repeats are dispersed by way of an RNA intermediate [2,3]. Consistent with this possibility, Alu family members are transcribed in vitro by RNA polymerase III (pol III) with the transcription start site corresponding to the 5' end of the consensus Alu family sequence [4]. Because the pol III promoter region is internal [5], newly inserted Alu family members should have sequences necessary for pol III transcription. Thus the numerical abundance of Alu repeats could result from the pol III promoter present within all member sequences [2,3].

The pol III promoter is necessary but not sufficient for transcription. 7S L RNA is ancestrally related to the human Alu sequence [29]. Ullu and Weiner have detected 7S RNA pseudogenes, which like Alu are flanked by short direct repeats,

polyadenylated on their 3' end and actively transcribed in vitro by pol III [6,7]. However, these pseudogenes do not code for the functional 7S RNA; the active gene includes upstream control elements which do not flank the dispersed 7S RNA pseudogenes [7]. Alu repeats, like 7S RNA pseudogenes, may be transcribed at a very low level or may even be transcriptionally silent. The clear sequence homology between 7S RNA and Alu repeats strengthens this inference.

The in vivo transcription of Alu family members is complex. Because Alu family members are broadly distributed throughout the human genome, they are present in other transcription units. Not surprisingly, as much as 10% of hnRNA consists of readthrough transcripts of both strands of Alu repeats [1]. Using an Alu family clone to hybrid select RNA, it was shown that 7S RNA is the only discrete length RNA which is homologous to Alu repeats [8]. However, individually promoted Alu transcripts might terminate at variable sites because each member is adjacent to entirely different 3' flanking regions. In this case human Alu family members could code for a collection of heterogeneous length pol III transcripts having fixed 5' ends. Consistent with this possibility, it was reported that the transcription of a nearby Alu family member is linked to the expression of a downstream epsilon-globin gene [9].

Assuming that Alu repeats are transcribed by pol III in vivo into heterogeneous length RNAs, primer extension by reverse transcriptase could reveal their common 5' transcription start site. Using this method we find that Alu repeats in HeLa cells are not generally transcribed by pol III.

MATERIALS AND METHODS

Cultured Cells

HeLa cells were grown in suspension in a spinner flask with Joklik's modified minimal essential media and 10% calf serum (GibCo). Cells were maintained at a cell density of $2-8 \times 10^5$ cell/ml.

Preparation of RNA from Cultured Cells

HeLa cells were washed twice in an equal volume of ice-cold RSB (150 mM NaCl, 1.5 mM MgCl₂, 1 mM KH₂PO₄, 10 mM Tris-HCl pH 7.6). Nuclear and cytoplasmic fractions were prepared by resuspending the final cell pellet at a density of 2×10^7 cell/ml in ice-cold RSB+NP-40 (RSB, 0.65% NP-40), followed incubating on

ice for 15 min. Nuclei were pelleted at 6000xg, 5 min., 4 °C. The cytoplasmic supernatant was removed and intact nuclei resuspended at 2×10^7 nuclei/ml in RSB+NP-40. Both nuclear and cytoplasmic fractions were extracted with an equal volume of 0.2 M Tris-HCl pH 7.6, 5 mM EDTA, 2% SDS, followed by sequential extractions with 1 volume phenol, phenol/chloroform, and chloroform. Nucleic acids were ethanol precipitated from the aqueous phase, then RNA was purified by pelleting in 5.7 M CsCl (Sigma-optical grade) at 100,000 xg, 20 °C, 24 hr. Nuclear and cytoplasmic RNAs were further fractionated on 15-30% sucrose density gradients containing 10 mM Tris HCl (pH 7.5), 1 mM EDTA, 0.1% SDS in a Beckman SW 50.1 rotor at 40,000 rpm for 2 hr. Twenty equal fractions were removed and characterized as follows: 1-7, >28 S RNA; 8-14, 18-28 S RNA; 15-20, <18 S RNA.

Electrophoresis and Hybridization of RNA

RNA was electrophoretically separated in a 1% agarose gel containing 6% formaldehyde in 20 mM NaHPO₄ (pH 7.0). The fractionated RNA was then transferred to nitrocellulose [10]. Filters were prehybridized and hybridized in 3 x SSC, 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% BSA, 0.1 % SDS, 200 ug/ml denatured salmon sperm DNA at 60 °C. Filters were washed in 3 x SSC, 0.1% SDS at 60 °C.

Permeabilization of HeLa Cells and Labelling of Nascent RNA

1.5×10^8 HeLa cells were pelleted at 3000xg, 5 min., 4 °C and washed 2x in 0.5 volumes ice-cold solution A (150 mM Sucrose, 80 mM KCl, 5 mM MgCl₂, 0.5 mM CaCl₂, 5 mM KHPO₄, 35 mM HEPES pH 7.4). The final pellet was resuspended in ice-cold solution A at a cell concentration of 1×10^8 cell/ml. 0.33 volumes ice-cold solution A + 0.4 mg/ml lysolecithin (Sigma) was added to the cells (final concentration 0.1 mg/ml lysolecithin) and incubated on ice 1 min. Cells were then pelleted at 3000xg, 5 min., 4 °C and resuspended in ice-cold solution A without lysolecithin at 1×10^8 cell/ml. Cells were diluted with 3 volumes solution B (150 mM Sucrose, 80 mM KCl, 5 mM MgCl₂, 0.5 mM MnCl₂, 35 mM HEPES pH 7.4, 2.5 mM ATP, 0.2 mM CTP, 0.2 mM GTP, 0.01 mM UTP, 25 ug/ml tRNA) and incubated at 37 °C for 5 min. If cells were to be treated with α -amanitin, 1/100 volume 2 mg/ml α -amanitin (P.L. Biochemicals) was added in the initial 5 min. 37 °C incubation. Labelling of nascent RNAs was initiated by the addition of 10 uCi α -³²P UTP per 5×10^7 cells. After incubation at 37 °C for 30 min. the reaction was stopped by the addition of 2 volumes 0.2 M Tris-

HCl pH 7.6, 5 mM EDTA, 2% SDS. The proteins were removed by sequential phenol/chloroform extraction of the aqueous solution followed by ethanol precipitation of the nucleic acids. The nucleic acids were resuspended in H₂O treated with 0.1% diethylpyrocarbonate (DEPC), and brought to 5.7 M CsCl (Sigma - optical grade). RNA was purified by pelleting at 100,000xg, 24 hr., 20 °C.

Construction of Single-Stranded Primers for Primer Extension

A 140 nt primer was constructed from the 3' end of the Alu family member BLUR 2 [11]. The Alu insert was released by Bam HI digestion and treated with bacterial alkaline phosphatase then end labeled with T4 polynucleotide kinase [10]. The 140 bp 3' end fragment was isolated following Alu I digestion and the labeled single-strand DNA was isolated from a strand separating polyacrylamide gel [12]. A 17 base oligonucleotide complementary to the Alu right monomer insert was chemically synthesized using a Systec automated DNA synthesizer. The sequence of the oligonucleotide is: 5' GCGATCTCGGCTCACTG-OH 3'

Primer Extension Analysis of RNA

5-10 ug RNA in 10.5 ul DEPC treated H₂O was added to 5 ul (5-50 pmole) T4 polynucleotide kinase labeled primer and the mixture heated to 80 °C for 3 min. 2.5 ul 10x reverse transcriptase buffer (0.5 M Tris-HCl pH 8.3, 0.1 M MgCl₂, 1.4 M KCl) was added and the solution in a beaker of hot water was slowly cooled to room temperature to anneal the primer. 2.5 ul 300 mM mercaptoethanol, 2.5 ul 10 x deoxyribonucleotides (10 mM each dATP, dCTP, dGTP, dTTP), and 1.0 ul RNasin (10 units - Promega Biotech) was added after cooling and the reaction initiated with 1.0 ul AMV reverse transcriptase (10-15 units - Life Sciences). Reactions were incubated at 37-45 °C for 30 min. then stopped by the addition of 50 ul phenol:chloroform (1:1). The aqueous phase was precipitated with ethanol, resuspended in formamide-dye mix and run on a polyacrylamide/7 M urea sequencing gel.

RESULTS

Transcription of Alu Sequences in Permeabilized Cells

Because Alu family members are transcribed in vitro by Pol III, nascent pol III transcripts from permeabilized tissue culture cells were examined for the presence of Alu sequences. Miller et.al. [13] report that Chinese hamster ovary (CHO) cells are permeabilized with lysolecithin at a concentration of

0.25 mg/ml and that RNA synthesis rates in permeabilized CHO cells are close to the in vivo rate. Human HeLa cells were treated as described by Miller et.al. at lysolecithin concentrations of 0.25, 0.1, and 0.025 mg/ml. Preliminary experiments using α - ^{32}P UTP as a tracer label show that cells treated with 0.25 mg/ml and 0.1 mg/ml lysolecithin synthesize RNA at high levels but the 0.025 mg/ml treated cells were less active. 0.1 mg/ml of lysolecithin was selected to permeabilize cells in subsequent experiments.

α -amanitin effectively inhibits RNA pol II transcription in permeabilized cells thus providing a means to examine pol III nascent transcripts. Cells treated with 2 ug/ml α -amanitin for 5 minutes prior to labeling for 30 minutes with α - ^{32}P UTP incorporate 80-90% less ^{32}P than untreated cells. Labelled RNA extracted from α -amanitin treated cells was compared to nascent transcripts from untreated cells by polyacrylamide gel electrophoresis (data not shown). tRNAs (pol III transcripts) are clearly labeled to the same extent in treated and untreated cells but other RNAs are not labeled in the presence of α -amanitin. Therefore, permeabilized HeLa cells are capable of RNA polymerase III transcription and RNA pol II transcription is effectively inhibited by exposure to α -amanitin.

In order to examine the strandedness of Alu homologous transcripts, single strand M13 clones were constructed to represent the two complementary strands of the Alu family member BLUR 2 [11]. Clone B2-1 represents the non-coding strand while B2-4 represents the complementary strand to the in vitro transcript. Nitrocellulose filters containing identical amounts of B2-1 or B2-4 DNA were hybridized to equal counts of RNA from amanitin or non-amanitin treated permeabilized cells. As seen in Table I, total transcripts in the absence of α -amanitin are symmetric with respect to either Alu strand, e.g. 357 cpm vs. 360 cpm in experiment 1. However, α -amanitin resistant transcripts hybridize with a considerable bias favoring clone B2-4 (complement strand), e.g. 144 cpm vs. 38 cpm in experiment 1. This bias is even more pronounced when the filter hybrids are digested with RNase A to remove any networking or secondary hybridization (experiment 2, Table I). The observed strand asymmetry of amanitin resistant transcripts suggests that sequences homologous to Alu family members direct pol III transcription. Because 7S RNA hybridizes to Alu repeats this

TABLE I - Transcription of Alu Sequences in the Presence of α -Amanitin^a

RNA ^b	B2-1 ^c (cpm)	B2-1+RNase (cpm)	B2-4 ^c (cpm)	B2-4+RNase (cpm)	B2-4 B2-1	B2-4+RNase B2-1+RNase
EXPERIMENT 1						
(-) α -amanitin	357	-	360	-	1.01	-
(+) α -amanitin	38	-	144	-	3.78	-
EXPERIMENT 2						
(-) α -amanitin	164	105	147	94	0.90	0.90
(+) α -amanitin	53	34	263	255	4.88	6.72

^aThe results of two separate experiments are presented. Background counts have been subtracted from each value reported. Experiment 1 background - 41 \pm 4 cpm, experiment 2 background - 22 \pm 3 cpm.

^bLabeled total RNA was made from HeLa cells permeabilized by lysolecithin. Prior to labeling cells were incubated with α -amanitin at 2 ug/ml which is sufficient to inhibit RNA pol II but not RNA pol III.

^c20 ug of the two M13 clones B2-1 and B2-4 were denatured by diluting into 100 ul 1.5 M NaCl, 0.5 M NaOH for 15 min. DNA was neutralized in 15 ml 20xSSC and bound to nitrocellulose by slow vacuum filtration. Filters were hybridized to 60,000 cpm (-) α -amanitin RNA or 50,000 cpm (+) α -amanitin RNA. Filters were washed in 3xSSC, 0.1% SDS, 60 °C and Cherenkov counted in a Beckman liquid scintillation counter. In experiment 2 filters were treated with 20 ug/ml RNase A for 30 min. at room temperature before counting.

bias might result from either 7S RNA transcription, Alu transcription, or a combination of both.

Analysis of total cellular RNA by Northern blots using both Alu strands as probes confirms that B2-4 correctly probes 7S RNA. As seen in fig. 1, both B2-1 and B2-4 hybridize to a smear of heterogeneous length transcripts probably representing pol II read-through products, but only B2-4 hybridizes to 7S RNA. Fig. 1 also confirms Weiner's observation that other than 7S RNA there are no discrete length transcripts that are homologous to human Alu repeats [8].

Detection of Alu Transcripts by Primer Extension

Is the observed asymmetry in Alu homologous pol III transcripts a result of 7S RNA or Alu transcripts? Several primers were designed for primer extension experiments to distinguish between 7S RNA and Alu promoted transcripts. Since Alu promoted pol III transcripts are predicted to have a distinct 5' end, these primers should also distinguish between specific Alu transcripts and other RNA molecules that contain Alu family members within their transcription unit.

The first primer tested was derived as a restriction fragment from the 3' end of a subcloned Alu family as described in Methods. Hybridization of this primer to 7S RNA would result

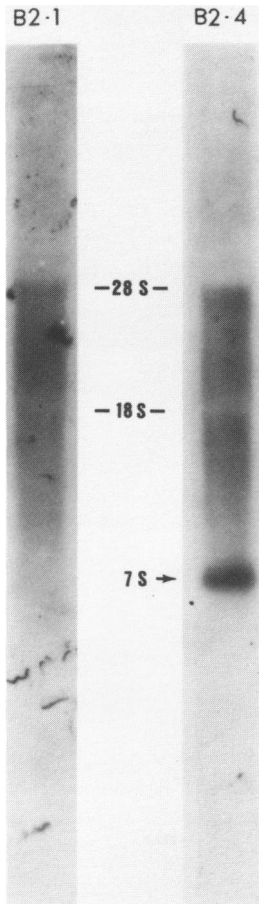


Figure 1. In Vivo transcripts of the Alu family. Duplicate northern blots of 3 ug HeLa nuclear RNA was hybridized to ^{32}P -labeled M13 clones B2-1 and B2-4 containing opposite strands of the 300 bp Alu family member BLUR 2. 28S, 18S, and 7S RNAs are indicated as molecular weight markers.

in a 185 nucleotides (nt) reverse transcript in the primer extension assay, whereas Alu promoted transcripts should result in a 300 nt long reverse transcript. As seen in fig. 2a, a signal of 185 nt corresponding to the predicted 7S RNA reverse transcript is seen at short exposure times in both cytoplasmic and nuclear RNA. An anomalous band of 180nt is also observed in the cytoplasmic fraction; the source of this band, which could be a 7S L RNA variant or a processed 7S RNA, was not investigated. The presence of 7S RNA in all gradient fractions is similar to Jelinek and Leinwand's finding that 4.5 S RNA, which is homologous to Alu and 7 S RNA, cosediments with longer hn RNA's [30]. At long exposure times a ladder of faint bands between 280 and 310 bp in length is barely detectable in nuclear

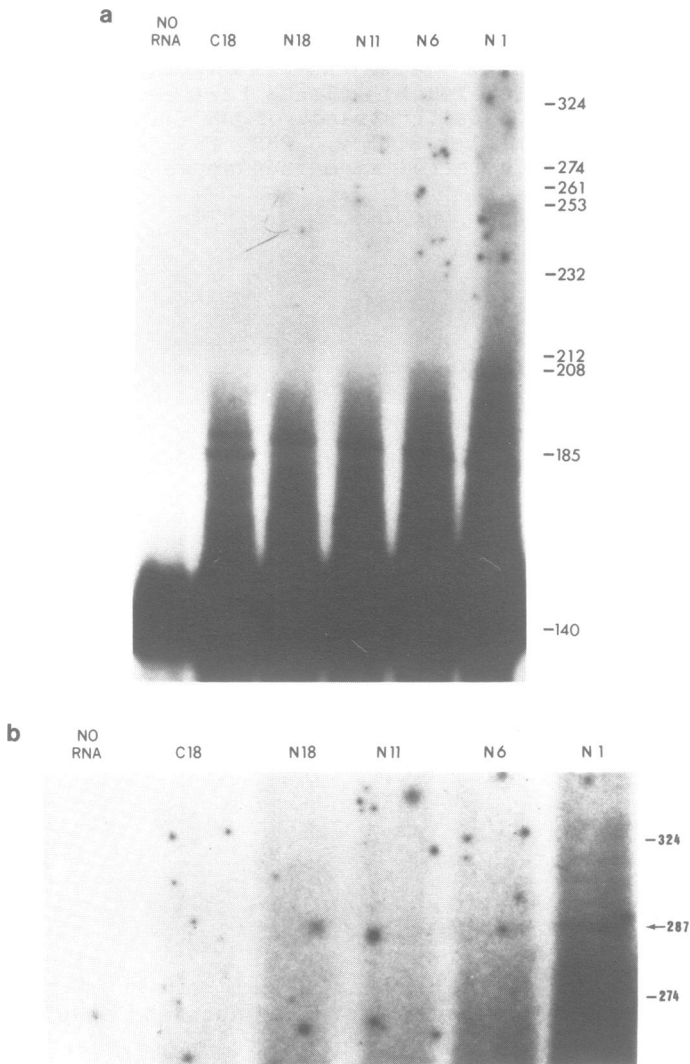


Figure 2. (a) Reverse transcripts of HeLa nuclear and cytoplasmic RNA using a 140 nt primer. 5 ug of HeLa nuclear and cytoplasmic RNA from sucrose gradient fractions was hybridized to 5 pmole of a 140 nt primer from the 3' end of the Alu family (see Methods), then reverse transcribed as in Methods and run on a 6% polyacrylamide sequencing gel. The lanes are as follows: No RNA (control), C18 (cytoplasmic RNA <18S), N18 (nuclear RNA <18S), N11 (nuclear RNA 18-28S), N6 (nuclear RNA >28S), N1 (nuclear RNA >28S). A strong band at 185 nt is indicated by an arrow. (b) A second polyacrylamide gel of the same fractions in fig. 2a exposed a longer time. Prominent bands are indicated by an arrow.

RNA but is absent in cytoplasmic RNA (fig. 2b). These faint bands approximate the length predicted for discrete Alu promoted transcripts, however, their length heterogeneity is unexpected since most genomic Alu family members share precisely the same 5' end with respect to the primer [4]. If the detected bands are reverse transcripts of Alu RNAs then additional primers which complement the Alu family sequence should confirm the existence of these Alu RNAs.

A synthetic oligonucleotide was prepared for use as a primer. This 17 nt long sequence is homologous to a 30 bp inserted sequence which is specific to the right half of the human Alu consensus and further does not complement 7S RNA (see Methods and fig. 3a). The 17mer should give a primer extension product of 240 nt if hybridized to an Alu promoted transcript.

Unexpectedly, the 17mer gives rise to a number of reverse transcript lengths including a prominent 78 nt long product (fig. 3b). Similar results were obtained by the use of two other Alu specific oligonucleotide primers which are not described here [31]. At least one of these bands results from a strong stop for reverse transcription due to RNA secondary structure rather than the 5' end of RNA molecules [31]. Consistent with this view, primer extension of the 17mer under more stringent conditions eliminates these background bands and gives rise to a distribution of reverse transcript lengths for nuclear RNA which are centered about the 240 nt size predicted for Alu promoted RNA (fig. 3c). Again we do not observe Alu size reverse transcripts for the cytoplasmic RNA (fig. 3c).

In summary, both the 17 nt Alu specific primer (fig. 3c) and the restriction fragment derived from a subcloned Alu repeat (fig. 2b) prime a distribution of reverse transcript lengths. The distribution of lengths (+10 nt) resulting from each primer is approximately centered on the predicted distance from the primer to the 5' end of the Alu consensus sequence. These Alu size reverse transcripts are observed for nuclear but not cytoplasmic RNAs. The good length agreement between the results for two different Alu specific primers supports the interpretation that these reverse transcripts are derived from Alu transcripts.

DISCUSSION

Nascent pol III transcripts from permeabilized HeLa cells show a 4-6 fold bias toward the in vitro sense strand of Alu

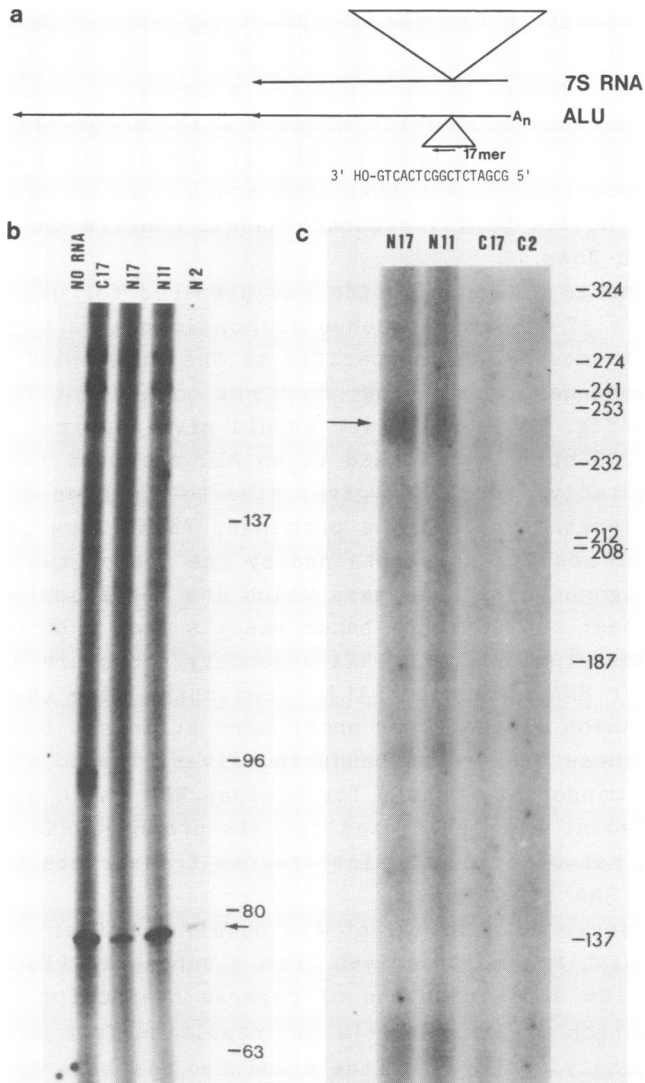


Figure 3. (a) The sequence and location of a 17 base oligonucleotide complementary to the Alu right monomer insert which is not complementary to 7S RNA. (b) Reverse transcripts of HeLa nuclear and cytoplasmic RNA using a 17 nt primer. 5 ug of HeLa nuclear and cytoplasmic RNA from sucrose gradient fractions was hybridized to 5 pmole of a 17 nt primer from the right monomer insert of the Alu family (see Methods), then reverse transcribed as in Methods and run on a 6% polyacrylamide sequencing gel. The lanes are as follows: No RNA (control), C17 (cytoplasmic RNA <18S), N17 (nuclear RNA <18S), N11 (nuclear RNA 18-28S), N2 (nuclear RNA >28S). A strong band at 78 nt is

indicated by an arrow. (c) Reverse transcripts of HeLa nuclear and cytoplasmic RNA using a 17 nt primer hybridized under stringent conditions. 5 ug of HeLa nuclear and cytoplasmic RNA from sucrose gradient fractions was hybridized to 5 pmole of the 17mer (fig. 3a), then reverse transcribed as in Methods using a modified 10 x reverse transcriptase buffer (250 mM Tris-HCl pH 8.3, 400 mM KCl, 100 mM MgCl₂) and run on a 6% polyacrylamide sequencing gel. The lanes are as follows: N17 (nuclear RNA <18S), N11 (nuclear RNA 18-28S), C17 (cytoplasmic RNA <18S), C2 (cytoplasmic RNA >28S). A set of bands centered on 240 nt is indicated by an arrow.

repeats (Table I). However, most of this asymmetric hybridization should be attributed to nascent 7S L RNA transcription and not pol III directed Alu transcripts.

Primer extension experiments reveal a barely detectable level of nuclear Alu transcripts which have a distribution of 5' ends centered about their predicted pol III transcription start site (figures 2 and 3). For simplicity of discussion we shall refer to such RNAs as "Alu RNAs". We believe that the primer extension method is very sensitive and that the very low level of Alu RNA detected here indicates that most Alu family members are transcriptionally silent. In support of this belief, it should be noted that reverse transcripts corresponding to 7S RNA are readily detected in our nuclear RNA preparations (fig.2). 7S RNA is cytoplasmic; nuclear 7S RNA is therefore only a minor subfraction of all 7S RNA. This subfraction could include nascent chains, molecules which shuttle between the nucleus and cytoplasm and molecules entering the nuclear fraction as an artifact of preparation. We estimate that the nuclear 7S fraction is at least 500 fold more abundant than the putative Alu RNA's. The basis of this estimate is the relative intensities of reverse transcripts corresponding to the putative Alu RNA (fig. 2B) and 7S (fig.2A) and the fraction of nuclear RNA (10%) represented in fraction N 1, which contains the putative Alu RNA's. The number of 7S molecules present in the nucleus is unknown and would certainly vary with different preparations. Comparisons of previous studies on the subcellular localization of various small RNA's suggest that 30,000 copies of 7S RNA might be present in the cell nucleus [14,15,16]. While we do not regard either this value of 30,000 copies nuclear 7S RNA or the ratio of nuclear 7S to Alu RNA, >500 to 1, as precise numbers, the inescapable conclusion is that there are very few copies of Alu RNA's in HeLa cells. This comparison of the relative

abundance of 7S and Alu reverse transcripts also assumes that their corresponding RNA's are primed with equal efficiency for reverse transcription. As the primer employed in the experiment of figure 2 is a restriction fragment of an Alu family member, we are confident that it will hybridize at least as well, if not better, to other Alu family members than it hybridizes to the more distantly related 7S RNA sequence.

Although a very small number of Alu transcripts do have 5' ends roughly corresponding to the predicted transcription start site of Alu we think it is unlikely that these molecules represent in vivo pol III Alu transcripts. Two independent primers demonstrate that these RNAs have heterogeneously positioned 5' ends which are merely distributed within +10 nt of the predicted pol III transcription start site (fig. 2b, 3b). Although the authentic Alu genes coding for these RNAs might have heterogeneously positioned 5' ends, we think that this is very unlikely. Of seventy sequenced human Alu repeats in the literature, most can be represented as having an exactly positioned (+1 nt) consensus 5' end that corresponds to the pol III transcription start site and only minor size variations due to 1 bp deletions or insertions [4,17]. Alternatively, the "Alu RNAs" may result from degradation products of hnRNA. Experiments of Jelinek and coworkers demonstrate the abundant interspersion of Alu sequences within hnRNA [1,18]. These sequences, which comprise as much as 10% of hnRNA, form RNase resistant double strands in vitro. It would not be surprising if a very small number of these same Alu transcripts formed duplex structures that resisted in vivo degradation. In this event in vivo degradation of hnRNA could result in Alu RNAs which are cleaved at a distribution of sites near their duplex ends. While not proven, this plausible explanation accounts for our finding of a low level of "Alu RNAs" having a distribution of 5' ends centered about the Alu consensus start site.

The present results contrast with Allan and Paul's report that an upstream Alu family member is transcribed in concert with the epsilon-globin gene [9]. The evidence for their findings is derived from an S1 protection experiment, using a unique 3' flanking probe to identify the termination sites of the putative Alu transcript. Assuming their result generally holds for all active genes, it is expected that thousands of Alu repeats should be transcriptionally active because Alu repeats are broadly

distributed near genes. As we have tested neither the particular cell line nor Alu repeat studied by Allan and Paul we do not necessarily discount their claim. However, the use of a flanking probe to test Alu transcription is an indirect assay and assumes the observed bands are due to Alu promoted transcripts. Alternatively, this Alu repeat could be part of a larger transcript in the opposite orientation to the epsilon-globin gene. Such antisense transcription occurs in the 5' regions of active genes [19] and even within actively transcribing genes [20].

The homology of the Alu family and 7S RNA indicates their ancestral relationship [6,8]. As mentioned in the Introduction the structure of 7S RNA pseudogenes resembles that of Alu repeats in all details. The finding that these 7S RNA pseudogenes do not code for an in vivo transcript strengthens the suggestion that most Alu family repeats are RNA pseudogenes. However, the argument that Alu repeats are RNA pseudogenes also implies that one or more human Alu repeats may code for an as yet unidentified gene product.

There are two families of short interspersed repeat sequences in rodents which, like human Alu repeats and 7S pseudogenes, are flanked by short direct repeats, polyadenylated on their 3' ends and transcribed in vitro by pol III. The B2 family sequences, which is closely related in sequence to tRNAs [21,22,27,28] is abundantly transcribed in vivo [23,24]. The B1 family, also called type I Alu equivalent sequences, are homologous to 7S RNA, rodent 4.5S RNA as well as the human Alu sequence [25]. Like their human Alu homologies the B1/type I-Alu rodent sequences are not transcriptionally active in vivo [25] (discounting their presence in hnRNA). However, an exogenous copy of a B1/type I Alu transfected into rodent cells is transcribed in vivo [26]. This implies that although B1/type I repeats are transcriptionally competent their in vivo transcription is efficiently repressed. Our inability to detect any transcriptional activity resulting from the 500,000 human Alu members in HeLa cells implies that the transcription of these sequences is similarly repressed.

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