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Cloning and characterization of cDNA copies of the 7S RNAs of HeLa cells

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ABSTRACT

We have cloned cDNA copies of *in vitro* adenylated 7S RNA of HeLa cells. The most representative clones in the library contain DNA fragments copied from the 7SL and 7SK small RNAs. The two classes of recombinants share no homology. The 7SL RNA contains at the 5' end of the molecule sequences homologous to the *Alu* sequence family. Hybridization to human genomic DNA shows that the 7SL and 7SK clones are homologous to two different families of repetitive sequences.

INTRODUCTION

A variety of cellular RNAs ranging in size from 4 to 8S are frequently referred to collectively as small cellular RNAs[1]. There are among these at least four 7S RNA species already described, two of which are located in the nucleolus[2]. The 7SL and 7SK (corresponding respectively to the ScL and SnK RNAs described by Zieve and Penman[3]) are usually extracted as part of the nucleoplasmic and cytoplasmic RNA of all cellular species examined up to now, although the 7SL seems to be essentially cytoplasmic, whereas the 7SK RNA is thought to be nuclear[3,2,4].

The two RNAs can account for 0.7-0.8% of the total RNA and their cellular content together with that of the 28S, 18S and 5S rRNA varies as a function of the total RNA[5]. Walker *et al.*[6] and more recently Gunning *et al.*[7] have shown that 7SL and K RNAs are associated with microsomal polysomes in L cells, HeLa cells, adult rat and rabbit tissues. The binding to polysomes occurs during the process of protein synthesis and is transitory.

Nothing is known about the function of the 7S RNA. Their high degree of conservation through evolution[8,6,2] combined with their abundance and presence in all cell types might suggest a structural function. More specifically Zieve and Penmann[3] and Zieve[1] have proposed a role of the 7SL RNA in the transport of mRNA from the nucleus into the cytoplasm.

7SL RNA is also present in fairly large amounts associated with genomic retroviral RNA. It is not clear whether it has a specific function during vi-

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ral infection although the amounts present in viral particles[9] suggest that it might not be a trivial contamination.

Recently Weiner[10] has shown that the 7SL RNA of HeLa cells has strong sequence homology with the Alu family of middle repetitive sequences found in human DNA[11] and therefore might derive from a subset of the Alu sequences.

This is consistent with the fact that the 7SL RNA is transcribed by polymerase III and Alu DNA constitutes a good in vitro promoter for polymerase III[12,13,14]. In analogy with this situation, the sequence of the 4.5S RNA isolated from rodents is homologous to that of the Alu family of DNA and is also transcribed by polymerase III[15].

The possibility of cloning cDNA copies of RNA molecules allows a detailed study of their structure and provides pure probes for the detection of the complementary RNA molecules in a complex population. Thus, it should be possible to distinguish different molecules of similar molecular weight, characterize precisely their location in the cellular context, and establish beyond doubt their possible relationship with known families of DNA sequences. In view of this we have constructed cDNA clones from in vitro polyadenylated 7S RNA of HeLa cells and we have characterized the recombinant molecules in relation to cellular RNA. In agreement with the data of Reddy et al.[2] we have found that the 7SK and 7SL RNA are two different molecular species, which do not crosshybridize with each other. The genomic representation of the two sequences is different and shows that both are derived from families of repeated DNA.

We also find that the sequence of 7SL RNA contains a region of homology with some of the available clones of Alu DNA and defines a new family of human repeated sequences which might be a subset of the Alu family of DNA.

MATERIALS AND METHODS

a) Purification of 7S RNA of HeLa cells

HeLa cells were grown in suspension culture in Eagle's minimal medium with Earle's salts, supplemented with 5% each of calf and fetal calf serum. Total cytoplasmic RNA was extracted from logarithmically growing cells as described by Perry et al.[16].

Poly(A)$^+$ RNA was purified by chromatography on oligo d(T) cellulose (Collaborative Research) and 7S RNA was isolated by repeated cycles of centrifugation on sucrose density gradients. The last step of purification was a 5-20% sucrose density gradient in 20mM NaAOC pH 5.5 spun for 20 hr at 40K in a Beckman rotor. 0.5 ml fractions were collected and an aliquot of each was electro-

phoresed on a 4% acrylamide gel in 7M urea. RNA was visualized by staining with 0.5 ug/ml ethydium bromide in water and fractions containing 7S RNA as the main component were pooled and precipitated with 3 volumes of ethanol.

b) Adenylation of 7S RNA

Adenylation of 7S RNA was carried out using E.coli poly(A) polymerase[17]. Following adenylation the enzyme was inactivated by addition of one volume of 0.2M EDTA pH 7.0 and poly(A) containing molecules were selected by chromatography on oligo d(T) cellulose. Usually 50% of the adenylated RNA was able to bind to oligo d(T) cellulose.

c) Cloning of 7S cDNA

cDNA was synthesized with the AMV reverse transcription using 7S RNA as a template and converted to the double stranded form with the Klenow fragment of DNA polymerase I from E.coli (Boehringer) according to Wickens et al.[18]. The yield of cDNA was in the range of 20 to 30% per ug of adenylated 7S RNA.

Double stranded cDNA was successively digested with the S_1 nuclease and ligated to EcoRI synthetic linker molecules (Collaborative Research). Cohesive ends were generated by digestion with the EcoRI restriction enzyme and these molecules were ligated to the λ641 cloning vector as described by Scherer et al.[19].

The hybrid DNA molecules were packaged in vitro using procedure of Scherer et al.[19]. The yield was about 500 recombinant phages/ng of double stranded cDNA.

Recombinant phages with a clear plaque phenotype were selected by plating on E.coli POP101 lyc 7[20].

d) Screening of the recombinant phages

Screening of the recombinant phage plaques was carried out according to Benton and Davis[21]. The conditions for plaque hybridization were as described by Maniatis et al.[22]. The recombinant clones were screened with ^{32}P labelled 7S cDNA and human DNA labelled with ^{32}P by nick translation. The phages from positive plaques were grown and the size of the recombinant DNA segments was determined. The recombinant phages containing the longest DNA inserts were further analysed after subcloning the insert in the EcoRI site of the pACYC184 plasmid[23]. Recombinant DNA work was carried out under P3 EK1 conditions.

e) Hybridization elution experiment

20 to 80 ug of human and plasmid DNA from various recombinant clones were loaded onto millipore filters as described by Melli et al.[24]. 7S RNA was labelled at the 3' OH end by ligation with ^{32}P-pCp (Amersham) using RNA ligase (PL Bio-

chemicals)[25]. Specific activities of 2 to $10x10^6$ cpm ug RNA were routinely obtained. ^{32}P labelled 7S RNA was hybridized to the DNA of different clones at a concentration of 1.0 ug/ml in 4xSSC at 68°C for 14 hours. After hybridization, the filters were washed in the hybridization buffer at 68°C until the background was negligible. Filters were then washed at room temperature in sterile water and the hybridized RNA was eluted by incubating the filters in 0.5 ml sterile water containing 10 ug/ml of carrier tRNA at 90°C for 5'. The eluted RNA was recovered by precipitation with 3 volumes of ethanol.

f) Partial T_1 digestion of purified 7S RNA species

The selected 7S RNA species were treated as follows. 6 ug of carrier tRNA were added to each sample and partial digestion with T_1 ribonuclease was carried out at 55°C for 12' in a buffer containing 33mM sodium citrate pH 5.0, 1.7mM EDTA and 7M urea. The amount of T_1 ribonuclease (PL Biochemicals) was chosen according to the instructions provided with the RNA sequencing kit of PL Biochemicals. A partial alkaline hydrolysis of one sample was also carried out in CO_3/HCO_3 buffer at 90°C for 6'. After digestion, the samples were chilled in ice and diluted twice with electrophoresis loading buffer.

g) Acrylamide gel electrophoresis in 7M urea

Acrylamide gel electrophoresis in 7M urea was carried out according to Maniatis et al.[26]. Before electrophoresis, the samples were resuspended in 99% formamide, 4% Ficoll and 0.05% each of Bromophenol blue and Xylene cyanol and heated at 90°C for 3'. The gels (400x200x0.5 mm) were run at 20mA (1500 to 2000 volts) and autoradiographed at -80°C using X-Ray Fuji films and Ilford intensifying screens.

h) Acrylamide-agarose gel electrophoresis in 2.2M formaldehyde.

The gel contained 2.5% acrylamide, 0.125% bis-acrylamide, 0.7% agarose and 2.2M formaldehyde in 20mM MOPS, (N-Morpholinopropanesulphonic acid) 5mM NaAOC, 1mM EDTA pH 7.0, which was also the electrophoresis buffer. Before loading, the samples were resuspended in 20ul electrophoresis buffer containing 50% formamide and 2.2M formaldehyde and heated at 60°C for 5 minutes. The gel (240x200x2 mm) was run at 50mA (200-300 volts) until the bromophenol blue reached the bottom of the gel. After electrophoresis, the gel was wahsed several times in water, dried onto a sheet of 3MM paper and autoradiographed at room temperature for several days.

i) Southern transfer and hybridization procedures

DNA from recombinant clones and human DNA from placenta were digested with restriction enzymes as described in the figure legends. The products of diges-

tion were fractionated on agarose gels and transferred to nitrocellulose filters according to Southern[27]. The hybridization conditions were as described for the plaque hybridization.

j) Source of enzymes

Polynucleotide kinase, T4 DNA ligase, E.coli poly(A) polymerase from E.coli and restriction enzymes were kindly provided by H. Cambier and V. Pirrotta (EMBL, Heidelberg).

RESULTS

a) Cloning of cDNA copies of 7S RNA

7S RNA was fractionated from the total cytoplasmic RNA of HeLa cells. Electrophoresis of the RNA from the final sucrose density gradient shows that certain fractions are clearly enriched for the K and L 7S RNA components described by Zieve and Penmann[3] (Figure 1A, fractions 6 and 7). 7S RNA was adenylated with the E.coli poly(A) polymerase using ATP as a substrate according to Sippel[17]. The conditions were such that on average, 25 to 50 adenine residues/molecule were added. This RNA was transcribed with AMV reverse transcriptase and the electrophoresis of the resulting cDNA in an acrylamide-urea gel is shown in Figure 1B. Two radioactive bands of the expected molecular weight were detected, suggesting that both RNA species might have been transcribed by the reverse transcriptase. The cloning of the double stranded cDNA was in the λ641 phage. Approximately 2000 phage recombinant plaques were screened with ^{32}P cDNA obtained from enriched 7S RNA. 80% of the clones gave positive signals.

Since the library should contain at least two types of recombinant DNAs, one derived from the 7SL DNA and homologous to Alu DNA and the other derived from 7SK RNA, we carried out a second screening using an Alu DNA probe. Human genomic DNA which contains approximately 5% Alu like sequences, seemed a convenient probe. 50% of the plaques gave a faint signal with the genomic DNA probe. Two types of clones were selected for further analysis: clones which hybridize exclusively to 7S RNA and clones which hybridize to 7S RNA and total human DNA. These two types of clones do not cross-hybridize as we have observed by subcloning the inserted fragment in the pACYC184 plasmid, and testing the recombinant phage DNAs with the plasmid subcloned (not shown).
The result suggests that the two types of cDNA recombinant phages are derived from two different 7S RNA molecules. The two classes of recombinant plasmids were tentatively called p7K and p7L: p7K are the the clones hybridizing only to ^{37}P 7S cDNA and p7L are those hybridizing also to human DNA.

Back hybridization of p7K and p7L DNA to the library of recombinant phages

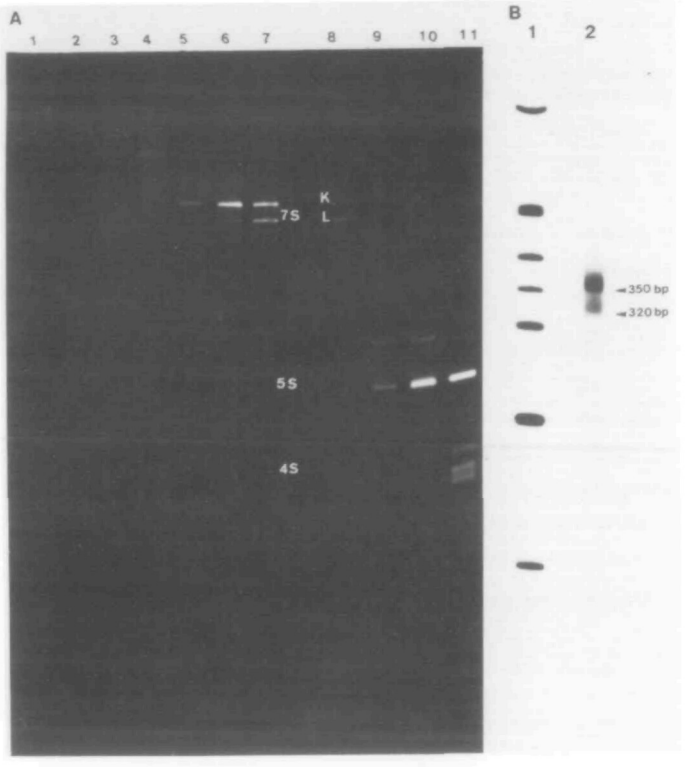

Figure 1: A. 4% acrylamide-urea gel electrophoresis of sucrose density gradient RNA fractions. The RNA from 1/10 volume of every fraction from the middle part of a 5-20% sucrose density gradient in NaAcetate pH 5.5, was alcohol precipitated and resuspended as described. Fractions 6 and 7, enriched for 7S RNA, were used for the cloning experiment. B. 5% acrylamide-urea gel electrophoresis of ^{32}P labelled cDNA. 1. ^{32}P-labelled pBR322 digested with the restriction enzyme HinfI as molecular weight marker. 2. ^{32}P cDNA synthesized using 7S RNA as a template.

shows that p7K is homologous to 30% and p7L to 50% of the phage plaques. This result confirms that the cDNA library contains essentially two types of cDNA sequences.

The restriction maps of the inserts in the p7K and p7L plasmids are shown in Figure 2. The p7L recombinant sequence is approximately 220 bp (bp=base pairs) from the 3' end of the RNA molecule, whereas the p7K DNA is 315 bp and therefore nearly full length. The restriction maps of the two recombinant DNAs are clearly different.

For reasons which are not clear, we were unable to obtain full length cDNA

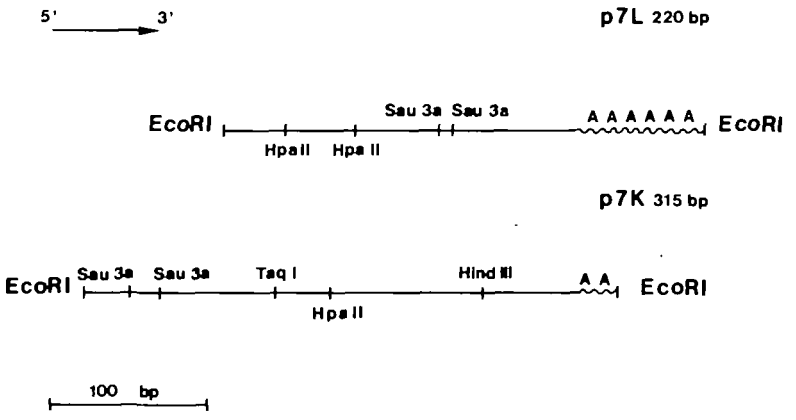

Figure 2: Restriction endonuclease cleavage sites of p7L and p7K DNAs. The polarity of the clones and the length of the poly(A) tails were determined by partial sequencing.

recombinant molecules of the 7L type from the phage recombinant library.

b) Relationship of the p7L and p7K recombinant fragments with cellular RNA

In order to identify the RNA molecules represented by the recombinant cDNA clones, we hybridized the p7K and p7L DNAs to a 7S rich RNA fraction from a sucrose density gradient, labelled at the 3' end with ^{32}P pCp.

The complementary RNA was eluted from the DNA and electrophoresed in a denaturing gel (Figure 3). Under these conditions, the RNA samples are resolved into one major band corresponding either to the L or K 7S RNA components described by Zieve and Penmann[3]. The low molecular weight faint bands migrating faster than 7S RNA are probably due to RNA degradation during the process of elution and they are practically absent in the control samples.

The RNAs complementary to the p7K and p7L DNAs are shown respectively in slots 1 and 5 and slot 3 of Figure 3. As compared with the controls (slots 4 and 7), the RNA complementary to p7K DNA migrates in the position of the 7SK RNA whereas the other RNA corresponds to the 7SL RNA. In order to see whether and which one of the RNAs was complementary to Alu DNA we have hybridized 7S RNA to Blur 8 DNA[28]. Blur 8 is a recombinant DNA plasmid clone containing an Alu like sequence which was kindly provided by T. Friedmann. The RNA eluted from Blur 8 DNA is shown in slot 8 and comigrates with the 7SL RNA in agreement with the data of Weiner[10]. It should be noted that the hybridization of 7S RNA to Blur 8 DNA was very inefficient as compared with that to the p7L

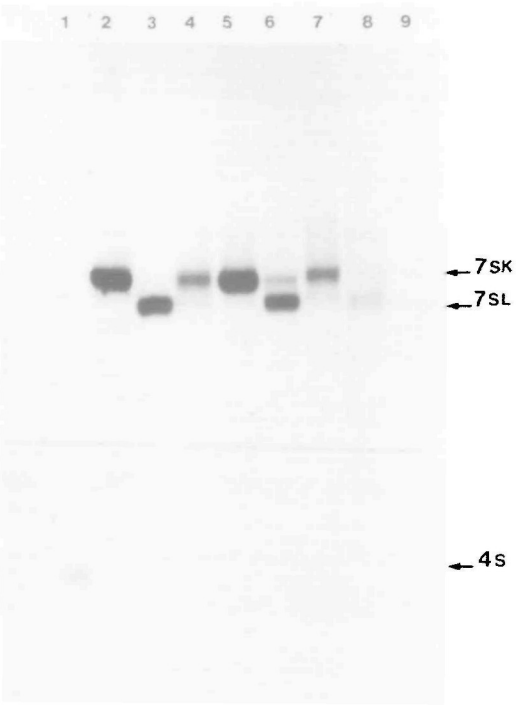

Figure 3: ^{32}P labelled 7S RNA was hybridized to 20 ug of recombinant plasmid DNAs or human placenta DNA, to 80 ug Blur 8 DNA, or to E.coli DNA. Aliquots of the RNA eluted from each RNA-DNA hybrid was electrophoresed on 2.5% acrylamide, 0.7% agarose, 2.2M formaldehyde gel as follows: 1/10 of the total RNA was loaded on the following slots: 2 and 5. RNAs eluted respectively from $p7K_1$ and $p7K_2$ DNAs ($p7K_1$ and $p7K_2$ are two independent recombinant plasmid DNAs complementary to 7SK RNA), 3. RNA eluted from p7L DNA, 6. RNA eluted from human placenta DNA. 1/2 of the total RNA was loaded on the following slots: 8. RNA eluted from Blur 8 DNA, 9. RNA eluted from E.coli DNA as a control of background. Track 1 contains ^{32}P labelled tRNA of HeLa cells; tracks 4 and 7 contain ^{32}P labelled 7S RNA. The exposure of the film was for 16 hr except for slots 8 and 9, which were exposed for a week.

clone or to human genomic DNA (slot 6). The comparison shows that at least 100x less RNA was bound by an amount of Blur 8 DNA 4x larger than that of the other DNA samples. This suggests that either the homology between 7SL RNA and Blur 8 DNA is weak or only a minority of the 7SL molecules are Alu like.

Slot 6 shows the pattern of migration of the 7S RNA which hybridizes to total genomic DNA. Two bands are visible, one slow and weak, comigrating with the 7SK RNA and the other faster and stronger which coincides with the 7SL RNA. This result indicates that the two RNA components derive from genomic multi-

gene families. A comparison of the intensity of the 7SL band in slots 6 and 8 clearly shows that the efficiency of hybridization of 7SL RNA to genomic DNA is several orders of magnitude higher than that to Blur 8 DNA, suggesting that the majority of the repeated DNA complementary to the 7SL RNA in the human genome is different from the Blur 8 sequence. These observations are consistent with the following hypothesis. The 7SL RNA population might contain two different types of sequences, one homologous to and the other different from Alu DNA. Alternatively, the RNA populations might be homogeneous and partially homologous to Alu DNA.

A partial characterization of the 7S RNA molecules hybridizing to the p7K, p7L and human genomic DNAs is shown in Figure 4. Gel electrophoresis of partial T1 digests of ^{32}P 7S RNA eluted from the RNA-DNA hybrids with the DNAs listed above shows that the banding patterns of the RNA complementary to the p7K and p7L DNAs are quite distinct and a mixture of these two patterns is recognizable in the RNA eluted from genomic DNA. In this track the pattern corresponding to the RNA from p7K is weaker than that corresponding to p7L DNA. This result confirms the experiment of Figure 3.

c) Relationship of 7SL RNA to Alu DNA

Since the hybridization-elution experiment indicates that the 7SL RNA component may contain two different types of sequences we carried out an experiment in order to find out whether or not these sequences are part of the same molecule.

7SL RNA eluted from a p7L DNA-RNA hybrid was hybridized to the 7L and 7K recombinant fragments and to Blur 8 DNA in addition to two different cloned Alu-like fragments, as described in the legend of Figure 5.
This hybridization (Figure 5B) is compared with that of the total 7S RNA to the same recombinant sequences (Figure 5A). Pure 7SL RNA hybridizes strongly to the p7L DNA and, with much lower efficiency to the three Alu like DNAs, giving a pattern similar to that obtained with total 7S RNA. This result shows that the same RNA molecules which are complementary to the p7L DNA sequence contain one or more region(s) of homology with the Alu family of repeated DNA.

Surprisingly the hybridization of the isolated p7L DNA insert labelled with ^{32}P to the same set of recombinant DNAs shows a positive signal only to the homologous DNA, even after one week exposure (Figure 5C). This result shows that the p7L DNA does not contain Alu like sequences capable of making a stable hybrid neither with Blur 8 DNA, nor with the two Alu like genomic recombinants isolated in our laboratory. Since the p7L insert is 220 bp long from the 3' end of the molecule and the 7SL RNA is approximately 300 bp long,

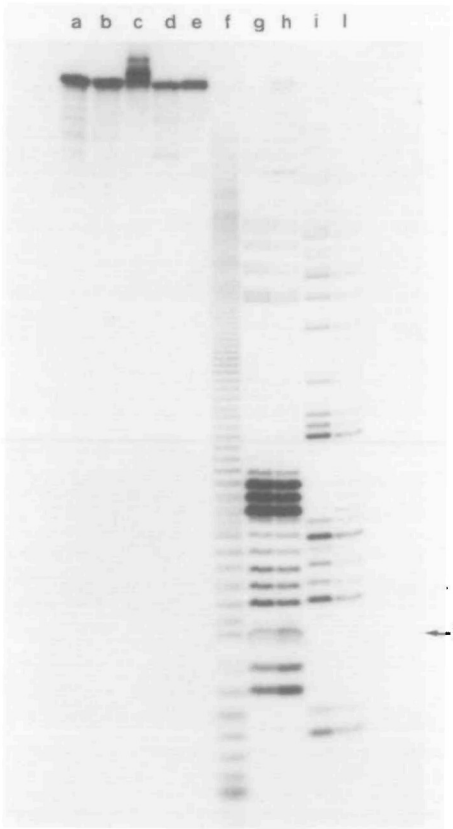

Figure 4: Partial T1 digestion of 7S RNA. The 7S RNAs eluted from RNA-DNA hybrid with p7K, p7L and human genomic DNA, were partially digested with T1 ribonuclease as described in Materials and Methods. The digestion products were electrophoresed on a 12% acrylamide, 0.6% bisacrylamide urea gel. a,b,d and e: undigested RNA respectively isolated with p7K1, p7K2, p7L and total human DNA. c: total ^{32}P 7S RNA. f: partial alkaline hydrolysis of RNA eluted from p7K1 DNA. g,h,i and l: T1 partial digests of RNA eluted from p7K1, p7K2, p7L and total human DNA.

the recombinant molecule is not complete and approximately 80 bp at the 5' end of the sequence is missing. We conclude that the 7SL RNA sequence capable of hybridizing to Alu DNA is restricted to the 5' end of the RNA.

d) Genomic representation of 7L and 7K recombinant DNA molecules

Figure 6A shows the hybridization of the p7L and p7K DNAs to a Southern transfer of EcoRI digested human genomic DNA. The hybridization with p7K DNA gives a complex array of bands which ranges from very high to low molecular weight,

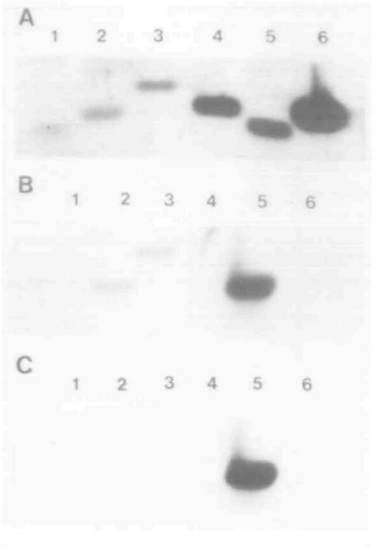

Figure 5: Hybridization of 7S RNA to Alu DNA. After digestion 1 ug of plasmid DNA with the restriction enzymes which excise the recombinant DNA fragment, the plasmid DNAs were electrophoresed on a 2.5% agarose gel. The DNA transferred on millipore filters was hybridized to A. total ^{32}P 7S RNA, B. ^{32}P 7S RNA eluted from a RNA-DNA hybrid with p7L DNA, C. pure p7L recombinant DNA fragment labelled with ^{32}P by nick translation. Tracks 1 and 3 contain two human recombinant DNA fragments, called respectively b6 and EB77, isolated from human DNA in our laboratory. By sequence analysis we have shown that these DNAs belong to the Alu family of repeated DNA, showing approximately 80% homology with the Alu consensus sequence of Deininger et al (1981), (Ullu and Melli, unpublished results). 2 contains Blur 8 DNA. 4 and 6 contain p7K1 and p7K2 DNA. 5 contains p7L DNA.

while p7L DNA shows a continuous pattern of hybridization which follows the optical density of the genomic DNA and is similar to the pattern obtained with Alu DNA.

A rough estimate of the reiteration frequency of the human genomic sequences complementary to the 7L and K inserts can be derived from the experiment shown in Figure 5B. Equal amounts of human placenta DNA spotted on millipore filters were separately hybridized to the p7L and K recombinant fragments labelled with ^{32}P by nick translation. The same probes were also hybridized to the λ7L and K recombinant DNAs (λ7L and K are the recombinant phages obtained from the cDNA library and complementary to the 7SL and K RNAs). The tracings of the hybridization spots allow a comparison of the amount of hybrid obtained with the different DNAs. Knowing the amount of recombinant DNA present in the λ phages, it is possible to calculate that the reiteration frequency of the 7L

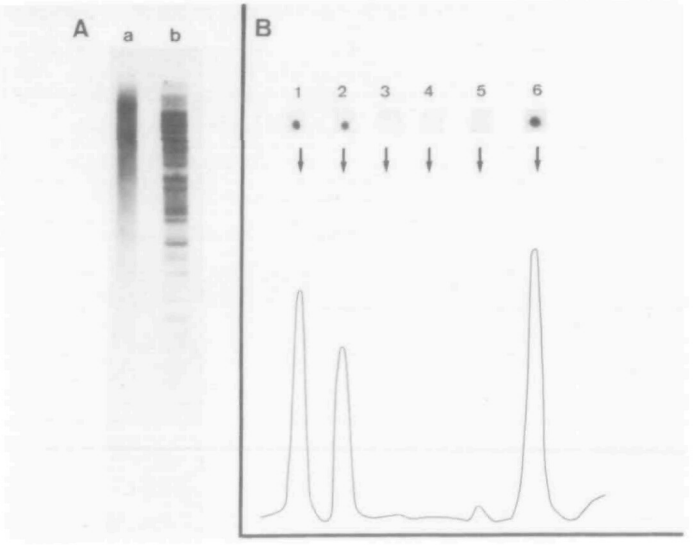

Figure 6: Hybridization of isolated p7L and K recombinant DNA fragments to human genomic DNA. A. Southern transfer. 10 ug of human placenta DNA were digested with the EcoRI restriction enzyme and electrophoresed on a 1% agarose gel. After transfer to millipore filter the DNA was hybridized to a: p7L DNA and b: p7K DNA inserts labelled with ^{32}P by nick translation. B. Spot hybridization. 1 ug of each type of DNA was loaded onto millipore filters and hybridized to ^{32}P labelled p7L (spots 1,2 and 3) and p7K (spots 4,5 and 6) DNA inserts. 1 and 4: λ7L DNA; 2 and 5: human placenta DNA; 3 and 6: λ7K DNA. The tracings corresponding to each hybridization are aligned with the respective spots.

and K like genomic sequences is respectively $5x10^4$ and $3x10^3$/haploid complement.

We conclude that both components of the 7S RNA are derived from repeated, possibly interspersed, sequences which belong to two different human "gene" or sequence families.

DISCUSSION

a) The cDNA library

The cloning of cDNA copies of poly(A)$^+$ RNA is a technique widely used in order to obtain large amounts of pure molecular species whose structure and relationship with cellular RNA and DNA can be easily characterized. The analysis of the cDNA library, carried out by back hybridizing the two recombinant DNA molecules to the λ phage plaques has shown that although the majority of the

recombinant clones contained 7S RNA copies, approximately 20% contained DNAs presumably derived from contaminant RNA molecules present in the total RNA population at low concentration. This is consistent with the relative purity of the 7S RNA used as a template for reverse transcription which shows only two major RNA components: the 7SL and K RNAs (Figure 1A). The two nucleolar 7S RNAs described by Reddy et al.[2] are not visible in the gel, in agreement with the cytoplasmic origin of the RNA. However, we cannot exclude that they might represent a minor proportion of the total RNA population, as a consequence of nuclear leakage.

We have shown that the two types of recombinant clones are different on several grounds. Both hybridize to the 7S cDNA probe, but only the λ7L plaques hybridize to human DNA. In agreement with this result, the two recombinant fragments are complementary to different RNA species (Figures 3 and 4) and hybridize to different sequences in the genomic DNA (Figure 6). Furthermore, the endonuclease restriction sites of the two recombinant molecules are not related (Figure 2). These results are in agreement with the fingerprint analysis of the 7SL and K RNAs, carried out by Reddy et al.[2] which shows that the two molecules are different.

Although unrelated, the two 7S RNA species seem to derive both from human multigene or sequence families (Figures 3 and 6). The experiment of Figure 6B indicates that the frequency distribution of the genomic sequences complementary to p7L DNA is at least one order of magnitude higher than that of the p7K like sequences. This result might explain why the screening of the library carried out with the human genomic DNA gave a positive signal only with the λ7L plaques. Probably, the concentration of the genomic DNA sequences complementary to the λ7K DNA, is not sufficient, under the conditions used for the plaque hybridization experiment, to allow detectable hybridization.

b) The homology of 7SL RNA with the Alu family of repeated DNA

The experiments that we have presented show that the 7SL RNA of HeLa cells contains a region of homology with Alu DNA at the 5' end of the molecule. A simple explanation of our finding, is that the 7S RNA derives from a composite DNA sequence, in which a short Alu fragment is joined to a different sequence, which is also repeated in the human genome (Figure 6A and B). We may, however, consider other possibilities. The detection of homologies between Alu DNA and the 7SL RNA is based on experiments of hybridization which require a rather good similarity of the two DNAs in order to give a stable hybrid structure. It is therefore possible that only the 5' end of the 7SL RNA is similar enough to Alu DNA to make a stable hybrid, whereas the remaining part of the molecule

has diverged considerably from what Deininger et al.[29] define the Alu consensus sequence. This might imply an evolutionary constraint on the 5' end of the 7SL gene(s), perhaps related to its function.

The third hypothesis that we might consider concerns the definition of the Alu family as such. We may envisage this family as being composed of a number of subsets, which have diverged in such a way as to change gradually from one type of sequence into a different one. Only the availability of a large amount of genomic sequences and accurate sequence comparison could clarify the relationship between the different classes of DNAs. Clearly the Alu-like clones so far analyzed, and sequenced, are relatively few, as compared with the genomic representation of Alu DNA and they might be typical of one subset of the Alu family. Similarly, the genomic sequence complementary to the 7SL RNA might represent another subset of the Alu DNA, which is as a whole, different from the dimeric structure shows for Alu-like sequences by Deininger et al.[29]. The length of the 7SL RNA (300 bp) which is the same as that of the known Alu DNA sequence and the polydispersed distribution in the human genome (Figure 6A) of the sequences complementary to it, support this hypothesis. It is in fact clear from our experiments, that the homology of the 7SL RNA with the "canonical" Alu sequence is weak, but the genomic hybridization of p7L DNA which does not contain the Alu like fragment, is very efficient. From the hybridization experiment (Figure 6B) we have calculated that the 7L DNA insert hybridizes to a family of sequences repeated approximately 5×10^4 fold, which is 1/10 of the value described for the Alu family of DNA.

In conclusion we would like to propose that the 7SL RNA is derived from one or more genomic sequences which might represent a subset of the Alu family of the human DNA. The structure of such sequences seems to be different from that of the Alu DNAs, cloned and analysed up to now.

The homology found at the 5' end of the 7SL RNA with Alu DNA is interesting in view of the fact, already mentioned, that the transcription of the 7SL RNA is Pol III dependent. Considering the good transcriptional properties of many of the available Alu like clones, we may think that the presence of such conserved sequences only at the 5' end of the molecule is related to the process of transcription.

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