Transcription of human 5S rRNA genes is influenced by an upstream DNA sequence

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ABSTRACT
Six human 5S rRNA genes and gene variants and one pseudogene have been sequenced. The six genes/variants were transcribed in a HeLa cell extract with about equal efficiency. Three genes contain the Sp1 binding sequence GGGCGG in position -43 to -38 and three genes contain the Sp1 like sequence GGGCGG in this position. The six genes contain furthermore one Sp1 binding site in a position about -245 and one ATF recognition site in a position about -202. A 12 bp sequence (GGCTCTTGGGGC) found in position -32 to -21 strongly influenced the transcriptional efficiency in vitro. This 12-mer, designated the D box, has also been found upstream a 5S rRNA gene from hamster and mouse. Removal of the Sp1 binding sites had no effect on the transcription in vitro whereas the transcriptional efficiency decreased to 10% if the D box was removed from the human 5S rRNA gene.

INTRODUCTION
The structural organization of 5S rRNA genes has been studied mainly in Xenopus and lower eucaryotes (Review 1,2). The role of the flanking regions in transcription has been studied and the importance of the 5'-flanking DNA sequence varies. The entire 5'-flanking region could be deleted from a Xenopus borealis 5S rRNA gene without affecting the transcription in vitro (3). Other studies demonstrated a 2-fold reduction in transcriptional efficiency after substitution of the nucleotides -11 to -16 upstream of this gene (4). An absolute requirement for 5'-flanking sequences has been demonstrated for the transcription of 5S rRNA genes from Drosophila melanogaster, Bombyx mori and Neurospora crassa (5,6,7). Information about the 5S rRNA gene structure and the transcriptional regulation in mammalian cells is scarce. Gene variants and pseudogenes were isolated from rat (8), mouse (9) and human cells (9–11). A bona fide gene was isolated from hamster cells and this gene was transcribed in a HeLa cell extract and in Xenopus oocytes as well (12). Interestingly one gene variant from rat was transcribed 10 times more efficiently than the bona-fide hamster gene in a HeLa extract, whereas other gene variants were either transcribed less efficiently, or not at all (8). A gene variant from mouse was transcribed in vitro while that from human cells was not (9).

A human 5S rRNA gene without a 5'-flanking sequence has been synthesized and it was efficiently transcribed in a homologous cell extract demonstrating that the coding sequence itself is sufficient for a certain level of transcription (13,14). Recently 5S rRNA genes were isolated from human cells and the organization and the repeat structure was characterised (15–17). In the present paper we have further studied the importance of the 5'-flanking sequence for the transcription of human 5S rRNA genes in vitro.

MATERIALS AND METHODS
Cloning
Human Placenta DNA was digested with restriction endonucleases SacI or BamHI or SacI + BamHI and then electrophoresed in 0.7% agarose gels. After excision of the 2.3 kb (SacI), 2.3 kb, 1.6 kb (BamHI) and 640 bp (SacI + BamHI) fragments, DNA was purified by electroelution, phenol extraction and precipitation from 0.7 M NH₄OAc by ethanol. DNA was ligated into Bluescript M13 plasmids and transformed into E.coli DH5α made competent with RbCl (18). Screening of transformants was carried out with [³²P]-UTP labelled RNA run-off transcripts synthesized by SP6 transcription of a mouse 5S rRNA gene variant (19).

Subclones were made from pHU5S3.1 (Fig.1), previously called pHU5S3-638 (17). The 5'-flanking sequence contain recognition sites for Apal, Smal, Fnu4HI and AccI (Fig.4A). Information about the 5S rRNA gene structure and the transcriptional regulation in mammalian cells is scarce. Gene variants and pseudogenes were isolated from rat (8), mouse (9) and human cells (9–11). A bona fide gene was isolated from hamster cells and this gene was transcribed in a HeLa cell extract and in Xenopus oocytes as well (12).

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Apal and the SmaI site was made by ExoIII/mung bean nuclease deletion (Fig.4A).

Transcription assay

The S100 extract was prepared from suspension cultures of HeLa cells as described by Dignam et al.(20). The protein concentration was 10 mg/ml. Transcription assays contained in a final volume of 20 μl: 10 μl extract, 5 mM MgCl₂, 85 mM KCl, 10% glycerol, 10 mM Hepes (pH 7.9), 0.5 mM ATP, GTP and CTP, 0.025 mM UTP, 10 μg C(α-32P)UTP, 0.3 mM dithiothreitol, 0.3 mM phenyl methyl sulfonyl fluoride, 0.1 mM EDTA and 0.5 μg DNA unless otherwise indicated. After incubation at 30°C for 60 min the reaction was terminated by addition of SDS (final conc 0.5%), E. coli t-RNA (final conc 200 μg/ml) and 80,000 cpm of marker 32P-RNA. The latter compound served as an internal marker to correct for any losses of material during extraction with phenol, precipitation with ethanol and electrophoresis. The marker RNA was made as a run-off transcript of the 3'-terminal SmaI-SacI fragment of clone pHU5S3.1. The purified RNA was electrophoresed in 10% polyacrylamide containing 7 M urea. Human 5S rRNA was used as a size marker. The transcription was measured by autoradiography. X-ray films were exposed for two or three different time intervals to ensure that comparison was made within the linear range and the X-ray films were then scanned in a Shimadzu dual-wavelength scanner. The nuclear extract was prepared as described previously (20).

Electrophoretic mobility shift analysis

A SmaI—SmaI fragment (pHU5S3.3) of pHU5S3.1 containing the 5S rRNA gene and 80 bp of the 5'-flanking and about 100 bp of the 3'-flanking sequence (Fig.1) was transcribed as efficiently as pHU5S3.1 in the HeLa S-100 extract described above. The fragment was isolated and labelled with 32P-dNTP in position —80 by Klenow polymerase. The electrophoretic DNA binding assay was performed as described by Fried and Crothers (21). HeLa extract (1 μl) was preincubated with either 300 or 500 ng poly(dl-dC) at 0°C for 20 min in 20 μl binding buffer D (25 mM Tris—HCl, (pH 7.9), 0.2 mM EDTA, 100 mM KCl, 0.5 mM phenyl methyl sulfonyl fluoride, 5 mM MgCl₂, 0.5 mM dithiothreitol, 20 mM Hepes (pH 7.9), 20% v/v glycerol). The endlabelled fragment was added and incubation continued for further 20 min. Samples were electrophoresed on 4% polyacrylamide gels in 90 mM Tris-Borate (pH 8.3) and the gels were transferred to 3MM paper and autoradiographed.

Footprint analysis

The endlabelled SmaI—SmaI fragment was incubated as described for electrophoretic mobility shift analysis (above). DNaseI or Micrococcus nuclease was added and the reaction mixtures were incubated for 1/2 min at 22°C. The reaction was terminated by addition of a stop solution (0.33 M NaAc, 50 mM EDTA) and the mixture was extracted with phenol-chloroform (1:1). DNA was precipitated with ethanol, purified and analyzed by electrophoresis on denaturing polyacrylamide gels (8% polyacrylamide, 6 M urea). Sequence ladders (G+A) were made from the labelled fragment according to Maxam and Gilbert (22) and used as markers.

Gel retardation assays were performed as described above and the radioactive bands were located by autoradiography. Free DNA and the retarded DNA/protein complexes were cut out and transferred to a buffer similar to binding buffer D except that it included DNaseI but no MgCl₂. After 30 min of preincubation at 25°C nonactivated DNaseI was allowed to penetrate the gel before MgCl₂ was added to a final concentration of 5 mM. The nuclease digestion was performed for 4 min at 37°C and the nuclease was then inactivated by addition of 0.5 M NH₄Ac, 0.1 M EDTA and 0.05% SDS. DNA was eluted at 37°C for 16 h, precipitated with ethanol, dissolved in 88% formamide and analyzed by electrophoresis on denaturing polyacrylamide gels.

Construction of transcription competitors

The plasmid pSV2CAT (23) which contains six SpI binding sites was digested with restriction enzymes AclI and HindIII. The mixture was electrophoresed on a 0.7% agarose gel, and after ethidium staining the 500 bp fragment containing the six SpI sites was isolated by centrifugation (24). This fragment was ligated into a Bluescript M13 plasmid.

The human 5S rRNA gene pHU5S3.1 was digested with BamHI and Accl and the mixture was electrophoresed on 0.7% agarose gels. A 273 bp fragment containing only the 5'-flanking sequence of the gene was isolated by centrifugation. After the XbaI and the Accl sites were made compatible with Klenow polymerase and the appropriate nucleotides, the 273 bp fragment was ligated into a Bluescript M13 plasmid.

Deletion of 5'-flanking sequence

The subclone pHU5S3.2 (Fig 4) was obtained from pHU5S3.1 by partial digestion with SmaI. The SmaI—SmaI fragment was removed and the plasmid containing the remaining SmaI—SacI fragment with the 5S rRNA gene was closed by ligation. pHU5S3.2 was opened by digestion with EcoRI, and after phenol extraction and ethanol precipitation, the DNA was dissolved in S1-nuclease buffer (50 mM NaAc (pH 5.7), 200 mM NaCl, 1 mM ZnSO₄, 0.5% glycerol). After preincubation at 37°C for 5 min a large amount of S1-nuclease (40—80 units per μg DNA) was added to ensure digestion of double stranded DNA. At different time intervals samples were removed and phenol extracted. DNA was precipitated with 0.7 M NH₄Ac and ethanol, purified and ligated by T4 DNA-ligase. The plasmids were transformed into competent E.coli DH5α cells and DNA isolated from the colonies and then sequenced. The subclone S-32 was obtained by this procedure and the subclones S-25, S-24, S-23 and S-21 were made from S-32 by a similar procedure.

RESULTS

Sequence analysis

Different DNA fragments containing human 5S rRNA genes and gene variants were isolated from genomic placenta DNA and the structure was characterized (17). The clones obtained are depicted in Fig. 1A and the derived subclones in Fig. 1B. The 640 bp BamHI—SacI fragments containing the 5S rRNA genes were sequenced and a comparison is shown in Fig. 2. Clone pHU5S6 is a 1600 bp BamHI—BamHI fragment and most likely it is not a part of the major 2.3 kb repeat cluster found in human DNA. pHU5S6 does not have a SacI site downstream from the termination sequence and apart from the 12 bp immediately downstream from the gene the downstream sequence differs completely from that of the other cloned genes. Few single basepair changes were observed between the six different BamHI—SacI fragments (pHU5S clones 1—6 in Fig.2). Three of the genes contain an upstream SpI binding sequence GGGCGGGGC in position —43 to —35 and in three genes the SpI like sequence GGGCGGGGC is found in this position. The six
genes contain furthermore one Spl binding sequence in a position about -245 and one ATF (activating transcription factor) recognition site in a position about -202.

A gene variant (pHU5S2) with an additional T nucleotide in position 47 in the internal control region and a pseudogene were also isolated. The pseudogene (pHU5S7) has completely different 5'-flanking sequences and the ATF recognition sites are shown. Numbers in parenthesis indicates the basepair number relative to the transcription start point.

**Figure 1.** Clones of 5S rRNA genes obtained from human placenta DNA (A) and derived subclones (B).

**In vitro transcription**

Transcription of the 5S rRNA genes in the different cloned fragments (Fig 1) were studied in a HeLa S-100 extract (Fig. 3). The 2.3 kb SacI fragment (pHU5S3) and the subclone pHU5S3.1 containing only the 640 bp BamHI—SacI fragment were transcribed with the same efficiency (Table 1). The two clones pHU5S1 and pHU5S2 were transcribed with a similar or a slightly higher efficiency than the pHU5S3 clones which have the Spl binding site. The two former clones have the Spl like binding site in position -43 to -35. A third clone pHU5S6 also possesses the Spl like binding site and this clone is transcribed with the same efficiency as pHU5S3. pHU5S4 and pHU5S5 are transcribed with the same efficiency as pHU5S3.2 and these clones contain the Spl like binding site in position -43 to -35. They lack the Spl like binding site in the position about -245 and the ATF (activating transcription factor) recognition site in the position about -202 and they have only a few bp substitutions in the flanking sequences (Fig. 2). The extra T nucleotide in the ICR region in pHU5S2 had no influence on the transcriptional activity.

The pseudogene which has a completely different 5'-flanking and 3'-flanking sequences and furthermore it contains 19 bp substitutions and one deletion within the coding region (Fig. 2).

Increasing parts of the 5'-flanking sequence were removed from pHU5S3.1 in order to study its importance in transcriptional regulation. The 5'-flanking region of this clone contains only 273 bp, 106 bp (Apal), 163 bp ("Exo") and 192 bp (SmaI) could be replaced by a plasmid sequence (Fig. 4A) without affecting transcription (Table 1). When 252 bp (Fnu4HI) were removed transcription decreased to 5-10% and without the 5'-flanking region (AccI) it was 1-5% of the original activity. The transcriptional efficiency of genes located in plasmids differs for supercoiled and for linear plasmids. As shown in Table 1 the transcription of 5S rRNA genes in linear plasmids is only 30-40% of the supercoiled DNA. The results obtained with
linear DNA showed the same pattern of transcriptional efficiencies as supercoiled plasmids. The DNA concentration used is optimal since no increase in activity was obtained by increasing or decreasing the DNA concentration. As opposed to the removal of 192 bp, removal of 252 bp from the 5'-flanking region of the 5S rRNA gene (pHU5S3.1, Fig 4A) had a profound effect on the transcriptional activity. By means of S1 nuclease digestion, several deletion clones were made between the Smal and Fnu4HI site. The sequences of these clones are shown in Fig. 4B together with the transcriptional efficiencies. The results show that a 12 bp sequence designated the D box is of major importance for the transcription. The plasmid sequence which substituted the D box did have some effect on transcription. This was shown using two clones with the same number of basepair deletions of the flanking sequence but with different plasmid basepair substitutions (S-21 and Fnu-21 in Fig.4).

The results demonstrate that the 5'-flanking region is important for transcriptional activity. It was not possible to abolish this effect in competition experiments when the 5'-flanking sequence (273 bp) was added in a 13 fold excess. The addition of a 500 bp fragment containing six Sp1 binding sites did not influence transcription (Table 1). The Sp1 binding sites were either added as an insert in a bluescript vector (3 fold excess in Sp1 binding sites) or as the 500 bp fragment (21 fold excess in Sp1 binding sites).

A human U3 RNA gene (25) was not transcribed in the HeLa S-100 extract used in the present experiments.

Protein binding experiments

The transcription of 5S rRNA genes in the HeLa cell extract does involve binding of proteins to the ICR region and probably also to the 5'-flanking region. However attempts to demonstrate such binding by gel retardation and footprinting were in vain. After incubation of a 5S rRNA gene with 5'-flanking sequence in a

### Table 1. Transcriptional efficiency of 5S rRNA genes and gene derivatives

<table>
<thead>
<tr>
<th>Clone</th>
<th>Transcriptional efficiency</th>
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<tbody>
<tr>
<td>pHU5S1</td>
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<td>-</td>
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<tr>
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<tr>
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<td>sc</td>
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<td>linear</td>
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<td>sc</td>
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<td>-</td>
<td>+ fragm. (6 Sp1)</td>
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<tr>
<td>5'-flank</td>
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<tr>
<td>Human U3 RNA gene</td>
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A HeLa S-100 extract (20 μl) was incubated with 0.5 μg DNA unless otherwise indicated and the state of the DNA is indicated as supercoiled (sc) or linear. The clones: pHU5S3.1, Apal, 'Exo', Smal, Fnu4HI and AccI are shown in Fig.4A. The fragment (6 Sp1) containing the six Sp1 binding sites was isolated as a 500 bp AccI–HindIII fragment from pSV2CAT (23) and cloned into a Bluescript plasmid (pKS). The competitors were added in the following amounts: 0.5 μg pKS (6 Sp1), 0.3 μg of the 500 bp fragment with the six Sp1 binding sites and 0.5 μg of the 5'-flanking sequence (273 bp) from pHU5S3.1.
HeLa S-100 extract no footprint could be demonstrated by DNasel or Micrococcus nuclease treatment. Gel retardation experiments were performed. Incubation was done in the presence of excess (500 fold) of poly dl-dC compared to the probe in order to bind unspecific DNA binding proteins. After electrophoresis of the mixture on a polyacrylamide gel three bands with a slower mobility than the unbound DNA resulted. The bands were isolated and DNasel treated but neither of these bands gave rise to any footprint.

**DISCUSSION**

RNA polymerase III synthesizes many species of small RNAs. These include 5S rRNA, t-RNA, 4.5S, 7SL, 7SK, U6, Alu, B1, H1 RNA (26), MRP RNA (27), Y3 RNA (28), BC1 RNA (29), VA RNA encoded by adenovirus and the EBERs encoded by Epstein-Barr virus (Reviews 2,30,31). Class III genes with an internal control region (ICR) are divided into two sets according to the structure of the ICR. 5S rRNA genes are the only members of type I and their promoters contain an intragenic A box and a C box. The type 2 promoters with an A and B box are found in genes encoding t-RNA, 7SL, VA RNA and the EBERs (31). The ICR in the 7SL gene differs somewhat from other type 2 genes and different regulatory sequences upstream to the genes have been demonstrated (32—33). Class III genes without functional intragenic control regions encode U6 and 7SK RNA and these genes contain TATA-like sequences and proximal sequence elements similar to the elements found in polymerase II transcribed genes (31).

Within the last few years the expression of 5S rRNA genes has changed from being solely a question of regulation from the internal control region to a possible additional involvement of regulatory sequences in the 5'-flanking sequences. Such regulatory mechanisms have been demonstrated in different lower eukaryotes (5,6,7) and Xenopus (34). Recently we have shown that the 5'-flanking sequence is important for an efficient transcription in vitro of the human 5S rRNA gene (17). It was therefore of interest to further study the effect of upstream sequence elements on the expression of 5S rRNA genes from human cells and compare this regulation with that in other mammalian cells.

We have isolated and sequenced six different human 5S rRNA genes/variants and one pseudogene (Fig 2). The six genes were transcribed equally well in a Hela S-100 extract whereas the pseudogene was transcribed with very low efficiency (Fig 3, Table 1). One gene variant contain an additional T nucleotide in position 47 in the ICR. Although this region binds TFIIIA (14) the extra T nucleotide did not affect transcription. The six 5S genes contain an Sp1 binding site in a position about —245 and three of the genes contain an additional Sp1 site in position —43 to —35. These Sp1 sequences have a high degree of homology with medium and strong binding sequences (35). The six genes furthermore contain an ATF recognition site in a position about —202. Several investigations have shown that some polymerase II and III promoters have similar regulatory sequence elements and that the three RNA polymerases can utilize common transcription factors (36-41). The Sp1 binding sites upstream of the 5S rRNA genes may be of importance for regulation of gene expression in vivo but no effect was seen in vitro. A similar conclusion was reached from competition experiments when excess Sp1 binding sequences were added to a transcription assay (Table 1). The 5'-flanking sequence was further analysed in the vitro transcription assay and the results showed that a 12-mer sequence upstream to the genes was important for the expression.

![Restriction map of pHU5.1 and pHU53.2](image-url)
of 5S rRNA genes. When this 12-mer sequence, designated the D box, was removed from a human gene the transcriptional efficiency decreased extensively (Fig.4). A similar result was obtained with a 5S rRNA gene isolated from mouse (42). The D box is located in position —32 to —21 in 5S rRNA genes of human origin and in position —33 to —22 in genes originating from mouse cells (42). In hamster cells (12) the D box is located in position —36 to —25 and further upstream the D box 6 bp are common for hamster and mouse cells (42). The slight differences in the position of the D box may explain the fact that the mouse gene is transcribed half as efficiently as a human gene in the HeLa S-100 extract (42). These results therefore suggest that species specific transcription factors exist in higher eukaryotes.

The binding of protein factors in the HeLa extract to the 5'-flanking region of 5S rRNA genes seems to depend on the presence of the 5S rRNA gene. This is concluded from the competition experiments. In the transcription assay the 5'-flanking DNA fragment (273 bp) was added in 13 fold molar excess without any inhibition of transcription of the 5S rRNA gene (Table 1). This suggests that binding of proteins to the D box is dependent on the presence of the 5S rRNA gene. The suggestion is in agreement with current hypothesis on the sequential binding of transcription factors in the transcription of yeast 5S rRNA genes. TFII A and C bind in the ICR region and they can then guide the binding of TFII B and polymerase III (43—45). TFII B bind to the upstream sequence in the region from about —43 to —10 (46).

The strict distinction between polymerase II and III transcribed genes is dissolving since it has been shown that both enzymes can utilize the same promoter sequences. Experiments have shown that the human oncogene c-myc can be transcribed by both polymerase II or III (47—49). Polymerase III can in addition utilize a large variety of different promoters depending on the gene to be transcribed. In the present paper we have shown that a D box upstream to the 5S rRNA gene is important for transcription. A search for the presence of this 12-mer in 270,000 sequences in the databases resulted in 22 positive and 20 of these were in mammalian DNA coding sequences. It was not possible to conclude about any possible function. We suggest that the D box, was removed from a human gene the transcriptional efficiency decreased extensively (Fig.4). A similar result was obtained with a 5S rRNA gene isolated from mouse (42). The D box is located in position —32 to —21 in 5S rRNA genes of human origin and in position —33 to —22 in genes originating from mouse cells (42). In hamster cells (12) the D box is located in position —36 to —25 and further upstream the D box 6 bp are common for hamster and mouse cells (42). The slight differences in the position of the D box may explain the fact that the mouse gene is transcribed half as efficiently as a human gene in the HeLa S-100 extract (42). These results therefore suggest that species specific transcription factors exist in higher eukaryotes.

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REFERENCES