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

Jens Nederby Nielsen+, Charlotte Hallenberg, Sune Frederiksen*, Pernille Dissing Sørensen§ and Bodil Lomholt

Department of Medical Biochemistry and Genetics, The Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark

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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 GGGCCG 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). It was furthermore demonstrated that removal of the 5′-flanking sequence from a human 5S rRNA gene resulted in a considerable reduction in transcriptional activity (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 NH_4OAc by ethanol. DNA was ligated into Bluescript M13 plasmids and transformed into *E.coli*/DH5α made competent with $RbCl_2$ (18). Screening of transformants was carried out with ^{32}P-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 ApaI, SmaI, Fnu4HI and AccI (Fig.4A). By means of these enzymes an increasing fraction of this region was substituted with plasmid sequences. One subclone ('Exo') with the 5′-flanking region preserved to a position between the

* To whom correspondence should be addressed

Present addresses: +Institute of Biological Chemistry B, University of Copenhagen, Sølvgade 83, DK-1307 Copenhagen K and §Biotechnological Institute, Lundtoftevej 100, DK-2800 Lyngby, Denmark

ApaI 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 $MgCl_2$, 85 mM KCl, 10% glycerol, 10 mM Hepes (pH 7.9), 0.5 mM ATP, GTP and CTP, 0.025 mM UTP, 10 μC_i (α-^{32}P)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 ^{32}P-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 gels 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 ^{32}P-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 dI-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 $MgCl_2$, 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 $MgCl_2$. After 30 min of preincubation at 25°C nonactivated DNaseI was allowed to penetrate the gel before $MgCl_2$ 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 NH_4Ac, 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 analysed by electrophoresis on denaturing polyacrylamide gels.

Construction of transcription competitors

The plasmid pSV2CAT (23) which contains six Sp1 binding sites was digested with restriction enzymes AccI and HindIII. The mixture was electrophoresed on a 0.7% agarose gel, and after ethidium staining the 500 bp fragment containing the six Sp1 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 AccI 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 AccI sites were made compatible with Klenow polymerase and the appropiate 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 $ZnSO_4$, 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 NH_4Ac 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 Sp1 binding sequence GGG-CGGGGC in position −43 to −35 and in three genes the Sp1 like sequence GGGCCGGGC is found in this position. The six

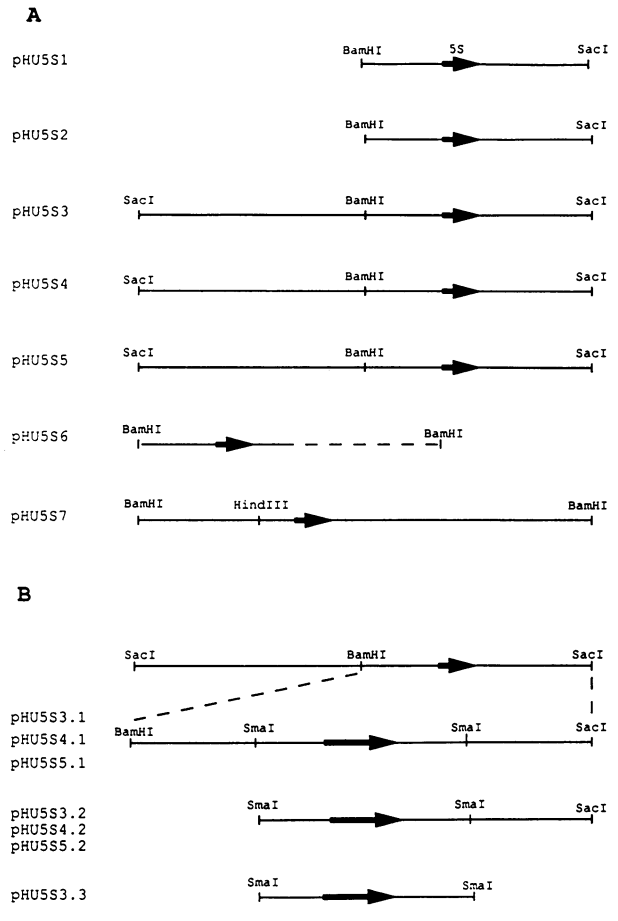

Figure 1. Clones of 5S rRNA genes obtained from human placenta DNA (**A**) and derived subclones (**B**).

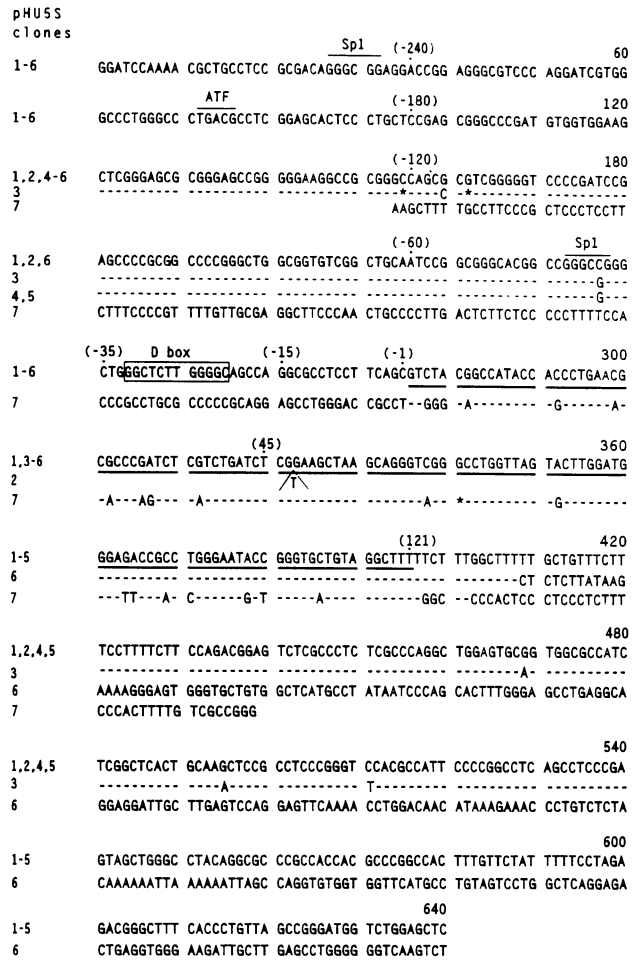

Figure 2. DNA sequences of different 5S rRNA genes. The BamHI−SacI fragments were obtained either directly from placenta DNA (pHU5S1 and 2) or derived from the SacI−SacI fragments (pHU5S3, 4 and 5). pHU5S6 was isolated as a 1.6 kb BamHI−BamHI fragment. It has no SacI site but the relevant 640 bp sequence is shown. The pseudogene pHU5S7 was isolated as a 2.3 kb BamHI−BamHI fragment and only part of the 5′-flanking and 3′-flanking regions have been sequenced. Deletions are indicated by an asteriks and the coding sequence is underlined. The D box is marked with a box and the Sp1 binding sequences and the ATF recognition sites are shown. Numbers in paranthesis indicates the basepair number relative to the transcription start point.

genes contain furthermore one Sp1 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 and 3′-flanking sequences and furthermore it contains 19 bp substitutions and one deletion within the coding region (Fig.2).

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 Sp1 binding site. The two former clones have the Sp1 like binding site in position −43 to −35. A third clone pHU5S6 also possesses the Sp1 like binding site and this clone is transcribed with the same efficiency as pHU5S3. pHU5S4.2 and pHU5S5.2 are transcribed with the same efficiency as pHU5S3.2 and these clones contain the Sp1 binding site in position −43 to −35. They lack the Sp1 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 sequence was transcribed with only about 5% efficiency (Fig.3).

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 (ApaI), 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 SmaI 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).

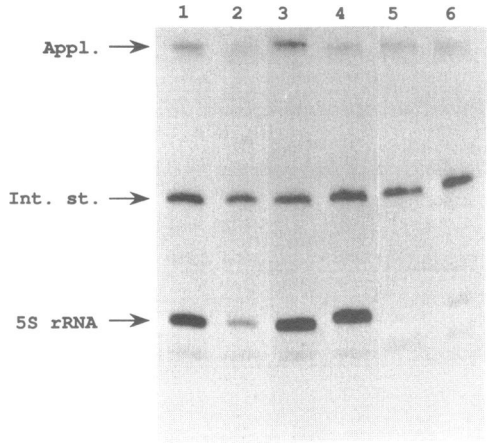

Figure 3. Transcription of 5S rRNA genes in a HeLa cell S-100 extract. pHU5S3.1, supercoiled (lane 1) and pHU5S3.1, linear (lane 2). pHU5S1, supercoiled (lane 3), and pHU5S2, supercoiled (lane 4). pHU5S3.1 deleted to −21 bp, Fnu-21, supercoiled (lane 5). The pseudogene pHU5S7 (5S pseudogene), supercoiled (lane 6). An internal standard (Int.st.) was added to the reaction mixture after end of transcription.

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

Clone			Transcriptional efficiency	
pHU5S1		sc	100−130	
−		linear	30−40	
pHU5S1.1		sc	100	
pHU5S2		sc	100−130	
−		linear	30−40	
pHU5S3	(2300 bp)	sc	100	
pHU5S3.1		sc	100	(0.5, 0.75, 1.0 μg)
−		linear	30−40	
pHU5S3.1	ApaI	sc	100	
−		linear	30−40	
−	'Exo'	sc	100	
−		linear	30−40	
−	SmaI	sc	100	(pHU5S3.2)
−		linear	30−40	
−	Fnu4HI	sc	5−10	
−	AccI	sc	1−5	
−	AccI	sc	1−5	(G in position +4)
pHU5S4.2		sc	100	
pHU5S5.2		sc	100	
pHU5S6	(1600 bp)	sc	100	
pHU5S7	(pseudogen)	sc	5−10	
pKS (6 Sp1)		linear	0	
fragm.(6 Sp1)		linear	0	
pHU5S1(sc)	+ pKS (6 Sp1)	linear	100	
−	+ fragm.(6 Sp1)	linear	100	
5'-flank		linear	0	(5'-flank pHU5S3.1)
pHU5S3.1(sc)	+ 5'-flank	linear	100	
Human U3 RNA gene		sc	0	

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, ApaI, 'Exo', SmaI, 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 DNaseI or Micrococcus nuclease treatment. Gel retardation experiments were performed. Incubation was done in the presence of excess (500 fold) of poly dI-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 DNaseI 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 1 and their promoters contain an intragenic A box and a C box. The type 2 promotors 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 promotors 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 in vitro transcription assay and the results showed that a 12-mer sequence upstream to the genes was important for the expression

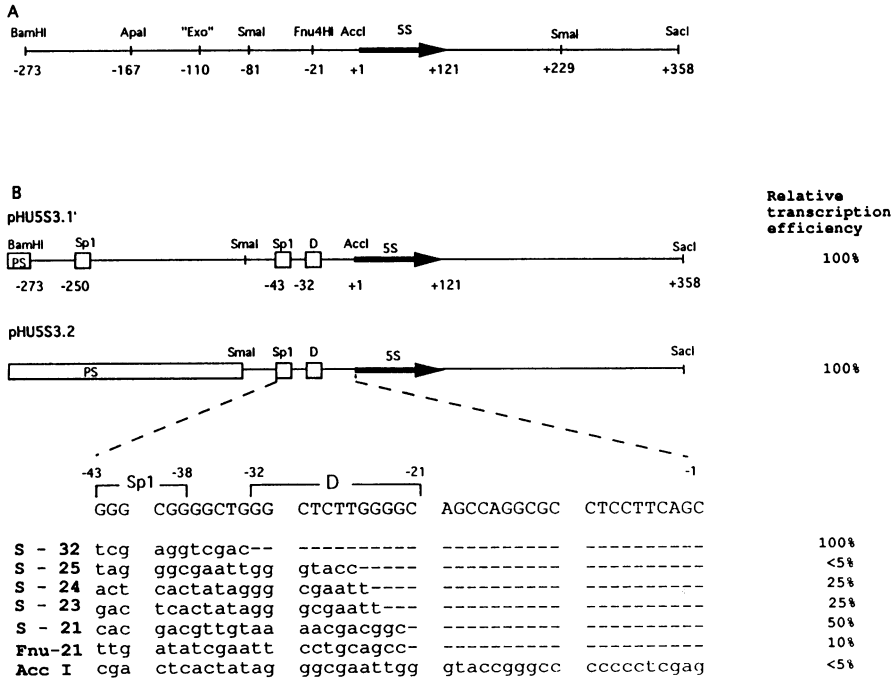

Figure 4. Restriction map of pHU5S3.1 (**A**). Different deletion derivatives and the corresponding in vitro transcription efficiency (**B**). The coding sequence is shown by an arrow. The plasmid sequence is indicated by PS or by small letters. The D box and the Sp1 binding sequences are indicated.

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 commen 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. TFIII A and C bind in the ICR region and they can then guide the binding of TFIII B and polymerase III (43−45). TFIII 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 promotors 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 (GGCTCTTGGGGC) is especially involved in transcription of mammalian 5S rRNA genes and together with the very conserved C nucleotide in position −1 it ensures a correct and efficient transcription initiation.

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REFERENCES

1. Korn, L.J. (1982) Nature 295, 101−105.
2. Geiduschek, E.P. and Tocchini-Valentini, G.P. (1988) Ann.Rev.Biochem. 57, 873−914.
3. Sakonju, S.,Bogenhagen, D.F. and Brown, D.D. (1980) Cell 19, 13−25.
4. Wormingon, W.M., Bogenhagen, D.F. and Brown, D.D. (1981) Cell 24, 809−817.
5. Sharp, S.J. and Garcia, A.D. (1988) Mol.Cell.Biol. 8, 1266−1274.
6. Morton, D.G. and Sprague, K.U. (1984) Proc.Natl.Acad.Sci.U.S.A. 81, 5519−5522.
7. Tyler, B.M. (1987) J.Mol.Biol. 196, 801−811.
8. Reddy, R., Henning, D., Rothblum, L. and Busch, H. (1986) J.Biol.Chem. 261, 10618−10623.
9. Emerson, B.M. and Roeder, R.G. (1984) J.Biol.Chem. 259, 7916−7925.
10. Arnold, G.J.,Kahnt,B.,Herrenknecht,K. and Gross,H.J. (1987) Gene 60, 137−144.
11. Doran,J.L.,Bingle,W.H. and Roy,K.L. (1987) Nucl.Acids Res. 15, 6297.
12. Hart, R.P. and Folk, W.R. (1982) J.Biol.Chem. 257, 11706−11711.
13. Wingender, E., Frank, R., Blocker, H., Wang, L., Jahn, D. and Seifart, K.H. (1988) Gene 64, 77−85.
14. Seifart, K.H.,Wang, L., Waldschmidt, R., Jahn, D. and Wingender, E. (1989) J.Biol.Chem. 264, 1702−1709.
15. Little, R.D. and Braaten, D.C. (1989) Genomics 4, 376−383.
16. Sørensen, P.D., Simonsen, H. and Frederiksen, S. (1990) Nucl.Acids Res. 18, 3060.
17. Sørensen, P.D. and Frederiksen, S. (1991) Nucl.Acids Res. 19, 4147−4151.
18. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning. A laboratory manual. Cold Spring Harbor Laboratory, New York.
19. Leah, R., Frederiksen, S., Engberg, J. and Sørensen, P.D. (1990) Nucl.Acids Res. 18, 7441.
20. Dignam, J.D.,Lebovitz, R.M. and Roeder, R.G. (1983) Nucl.Acids Res. 11, 1475−1489.
21. Fried,M. and Crothers,D.M. (1981) Nucl.Acids Res. 9, 6505−6525.
22. Maxam,A.M. and Gilbert,W. (1977) Proc.Natl.Acad.Sci.USA 74, 560−564.
23. Gorman,C.M. (1985) In Glover,D.M.(ed.),DNA Cloning−A practical Approach.IRL Press,Oxford,Vol.II,pp 143−190.
24. Heery,D.M.,Gannon,F. and Powell,R (1990) TIG 6, 173.
25. Yuan, Y. and Reddy, R. (1989) Biochem.Biophys.Acta 1008, 14−22.
26. Hannon,G.J.,Chubb,A.,Maroney,P.A.,Hannon,G.,Altman,S. and Nilsen,T.W. (1991) J.Biol.Chem. 266, 22796−22799.
27. Yuan,Y. and Reddy,R. (1991) Biochem.Biophys.Acta 1089, 33−39.
28. Kelekar,A. and Keene,J.D. (1990) Mol.Biol.Rep. 14, 173−174.
29. Tiedge,H.,Fremeau,R.T.Jr.,Weinstock,P.H.,Arancio,O. and Brosius,J. (1991) Proc.Natl.Acad.Sci.USA 88, 2093−2097.
30. Jelinik,W.R. and Schmid,C.W. (1982) Ann.Rev.Biochem. 51, 813−844.
31. Kunkel,G.R. (1991) Biochem.Biophys.Acta 1088, 1−9.
32. Bredow,S.,Kleinert,H. and Benecke,B-J. (1990) Gene 86, 217−225.
33. Bredow,S.,Surig,D.,Muller,J.,Kleinert,H. and Benecke,B-J. (1990) Nucl.Acids Res. 18, 6779−6784.
34. Oei,S-L. and Pieler,T. (1990) J.Biol.Chem. 265, 7485−7491.
35. Kadonaga,J.T.,Jones,K.A. and Tjian,R. (1986) TIBS 11, 20−23.
36. Howe,J.G. and Shu,M-D. (1989) Cell 57, 825−834.
37. Sawodogo,M. and Sentenac,A. (1990) Ann.Rev.Biochem. 59, 711−754.
38. Lobo,S.M.,Tanaka,M.,Sullivan,M.L. and Hernandez,N. (1992) Cell 71, 1029−1040.
39. Comai,L.,Tanese,N. and Tjian,R. (1992) Cell 68, 965−976.
40. White,R.J.,Jackson,S.P. and Rigby,P.W.J. Proc.Natl.Acad.Sci.USA 89, 1949−1953.
41. Taggert,A.K.P.,Fisheer,T.S. and Pugh,B.F. (1992) Cell 71, 1015−1028.
42. Hallenberg,C.Nederby Nielsen,J. and Frederiksen,S. Results to be published.
43. White,R.J. and Jackson,S.P. (1992) 71, 1041−1053.
44. Simmen,K.A.,Bernues,J.,Lewis,J.D. and Mattaj,I.W. (1992) Nucl.Acids Res. 20, 5889−5898.
45. Palmer,J.M. and Folk,W.R. (1990) TIBS 15, 300−304.
46. Kassavetis,G.A.,Braun,B.R.,Nguyen,L.H. and Geiduschek,E.P. (1990) Cell 60, 235−245.
47. Chung,J.,Sussman,D.J.,Zeller,R. and Leder,P. (1987) Cell 51, 1001−1008.
48. Bentley,D.L.,Brown,W.L. and Groudine,M. (1989) Genes & Devel. 3, 1179−1189.
49. Sussman,D.J.,Chung,J. and Leder,P. (1991) Nucl.Acids Res. 19, 5045−5052.