**Nucleic Acids Research**

## Supercoil induced S1 hypersensitive sites in the rat and human ribosomal RNA genes

István Financsek, László Tora, Gabriella Kelemen and Egon J.Hidvégi

'Frédéric Joliot-Curie' National Research Institute for Radiobiology and Radiohygiene,
H-1775 Budapest, Hungary

ABSTRACT

Rat and human ribosomal RNA gene fragments in supercoiled plasmids were examined for S1 nuclease hypersensitivity. In the transcribed portion of genes the number and distribution of S1 sites were found to be species specific. No S1 sites were detected in the promoter regions. In the nontranscribed spacer (NTS), downstream of the 3' end of 28S RNA gene, S1 sites appear to be conserved in rat and human rDNAs.

A rat NTS fragment (2987 nucleotides long), containing three S1 sites was sequenced and the S1 sites in this region were localized in polypyrimidine.polypurine simple repeat sequences. Other types of simple sequences, two type2 Alu repeats and an ID sequence were also found in the sequenced region. The possible role of simple sequences and S1 sites in transcription and in recombination events of rDNA is discussed.

INTRODUCTION

Transcriptionally active chromatin is often, but not always, characterized by the presence of DNAse I and/or S1 nuclease hypersensitive sites (SHS) in the 5' or 3' end of genes, transcribed by RNA polymerase II (reviewed in 1) and III (2). The DNAse I and S1 hypersensitive sites in many cases map very close to each other, or are overlapping (3, 4, 5) and the two enzymes may recognize different aspects of the same structure (6). The SHS detected in chromatin were also detectable in most, but not in all, cases in supercoiled plasmids (3, 6, 7). In the formation of the nuclease hyper- sensitive sites in plasmids or in chromatin, DNA conformation changes (7, 8) specific proteins (9, 10) or the torsional stress present in activ chromatin (11) may be involved. Although the DNAse I and S1 sites were often correlated with the expression state of genes, many of them are believed to be

involved in processes other than transcription (e.g. 12, 13, 14).

DNAse I hypersensitive sites in rDNA chromatin, probably correlated with gene activity, have already been detected (15, 16, 17). To date SHSs in rDNA fragments in supercoiled plasmids only of three Drosophila species have been examined (18). The conservation of S1 sensitivity, that was detected in Drosophila NTS regions that are divergent in sequence, might indicate the conservation of a specific function, this function, however, is not clear (18).

Neither DNAse I nor S1 hypersensitivity of mammalian rDNAs have yet been examined. In the present work rat and human rDNA fragments in supercoiled plasmids were examined for S1 hyper-sensitivity. SHSs in the NTS of the two genes, downstream of the 28S RNA genes seem to be conserved, as in Drosophila (18). A species specific distribution of SHSs in the transcribed part of the genes was also observed. No SHS were found at or near the promoter regions. The nucleotide sequence of a ~3 kb rat NTS 3' end fragment, that contains three SHSs was determined and found to contain a number of different simple repeat sequences. The possible involvement of these sequences in recombination processes is discussed.

## MATERIALS AND METHODS
### Materials

Restriction endonucleases, S1 and Bal31 nuclease were purchased from Bethesda Research Laboratories and were used according to the supplier's recommendation.

Plasmid DNAs were prepared according to the method of Birnboim (19).

E.coli DNA polymerase was supplied by Boehringer-Mannheim, $\alpha$-$^{32}$P-dATP by the Institute of Isotopes, Hungary, and the nick translation was made by the method of Rigby (20). $\gamma$-$^{32}$P-ATP and $\alpha$-$^{32}$P-dCTP were obtained from Amersham. The DNA sequencing was performed as described by Maxam and Gilbert (21).

### S1 nuclease digestion

Plasmid DNAs were digested with S1 nuclease essentially as described by Larsen and Weintraub (3). One unit of S1 enzyme

was used per microgram DNA and the digestion was performed in high salt buffer, containing 300 mM NaCl, 30 mM Na-acetate, pH 4.5, 3 mM $ZnCl_2$ and 0.1 mM EDTA, or in low salt buffer containing 30 mM NaCl, 30 mM Na-acetate, pH 4.5, 3 mM $ZnCl_2$, 0.1 mM EDTA at 25 or 37$^{\circ}$C for 15 min. The samples after S1 digestions were extracted with phenol, several times with chloroform, precipitated with ethanol and then digested with restriction enzymes. Bal31 nuclease digestions were carried out as described (18).

## Hybridization with nick translated probes

Hybridizations were performed in 3 x SSC (1 x SSC is 0.15 M NaCl, 0.015 M Na-citrate), 0.1% SDS, 20 mM Tris, pH 7.4, 1 mM EDTA, 10 x Denhardt's solution (1 x Denhardt solution is 0.02% Ficoll, 0.02% Bovine serum albumine, 0.02% Polyvinyl pyrollidone), 200 μg/ml carrier DNA, at 65$^{\circ}$C for 12-14 hours. The filters were washed with several changes of 0.1 SSC, 0.1% SDS at 65$^{\circ}$C for 3 hours.

## rDNA clones

The pNH10, p4.8 and p5.6 rat rDNA EcoRI clones were described previously (22, 23). The pNH8 SalI clone has recently been isolated. The map position of the clones in the rat rDNA repeat are shown in Fig.1.

Human rDNA clones used in this study were isolated by Wilson et al. (24).


## RESULTS

### S1 hypersensitive sites in rat rDNA clones

In supercoiled p4.8 plasmid, containing the promoter region and the 3' end of NTS of rat rDNA, three SHSs were detected (Fig.2A). In the samples without S1 treatment, only the bands expected from the restriction map were seen (Fig.2A lanes 5, 6). The three prominent bands, resulted from the S1 digestion indicated the presence of three major S1 cutting sites in super-coiled p4.8 plasmid (Fig.2A lanes 7-14). The positions of these S1 sites were also determined in the p3.3 and pNH10 plasmids (not shown). From the data obtained in these experiments the S1 sites were localized at 1.2, 1.6 and 2.1 kb from the EcoRI site in p4.8 i.e. -2.7, -3.2 and -3.6 kb upstream of the
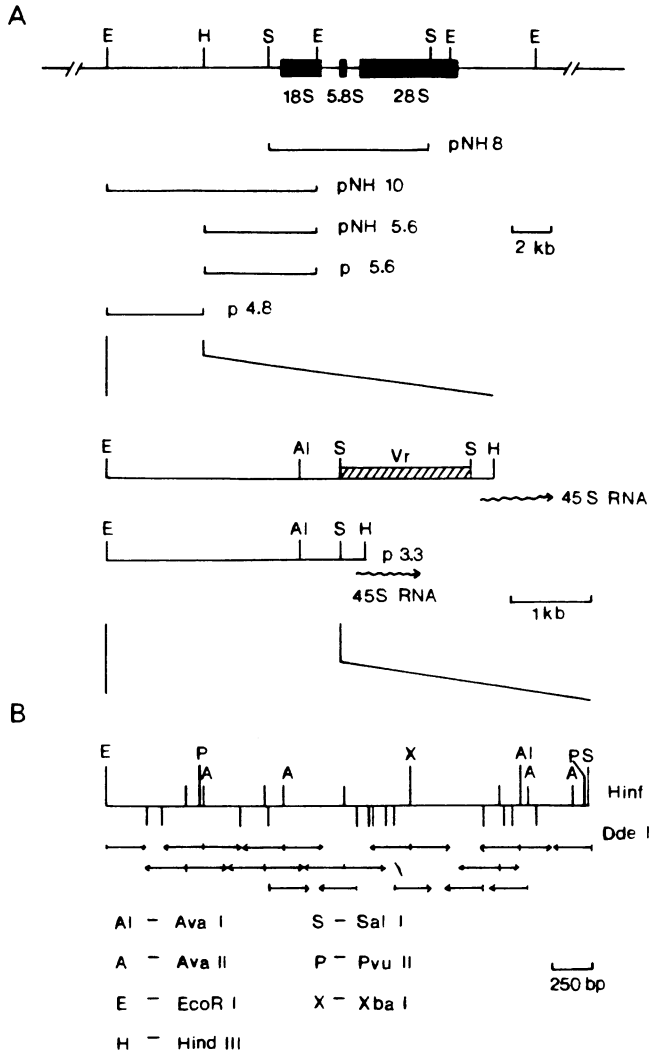
**Figure 1. Sturcture of the rat rDNA repeat and map position of clones used in this work**
A. The pNH10, pNH8 and pNH5.6 are Novikoff hepatoma rDNA clones and p4.8 and p5.6 are rat liver rDNA clones (22, 23). From p4.8 the variable region (Vr-hatched box) was deleted to obtain p3.3.
B. Restriction map and sequencing strategy (arrows) of the EcoRI/SalI rat NTS fragment. From the HinfI and DdeI sites the sequencing was performed on both DNA strands.

transcription initiation site. In the promoter region no S1 cutting occurred. The lack of SHS in the promoter region was not the result of masking effect of the strong SHSs in the NTS,

since S1 cutting could not be demonstrated in a HindIII/AvaI
subclone, containing only the promoter and variable regions
(not shown).

We examined the supercoiled p5.6 and pNH5.6 plasmids
containing the whole ETS and 1.7 kb of the 18S RNA coding
region for S1 sensitivity. Two S1 sites were detected in these
plasmids near the 5' end of the 18S RNA gene in the ETS. Since
the pNH8 plasmid overlaps with p5.6 and pNH5.6 in the region
that contains the S1 sites, only the results obtained in pNH8
are shown (Fig.2B). The two S1 sites in the ETS were 1.7 and
2.0 kb from the EcoRI site. In the rat ETS there is a
polypyrimidine tract, containing multiple CT repeats just
upstream of the 5' end of 18S RNA gene (25). Because of the low
resolving power of agarose gel ($\pm$50 nucleotides) we analyzed
the digestion products in polyacrylamide gel. The SalI site in
pNH8 is 450 bp from the 5' end of 18S RNA gene. In S1 treated
pNH8 plasmid the SalI digestion generated 430-440 bp fragments
(Fig.5B) indicating that S1 cutting in the ETS occurred in the
polypyrimidine tract at the 5' end of the 18S RNA gene (site 5
in Fig.6).

In pNH8 plasmid besides the two SHSs in the ETS there were
additional S1 sites (Fig.2B lanes 10, 11, 12) in the internal
transcribed spacers. Both ITS 1 and 2 in rat rDNA contain a
number of polypyrimidine.polypurine sequences (26). One of them
starts immediately upstream of the 5' end of 28S RNA gene. It
is possible that S1 cutting in ITS 1 and 2 occurred in the
polypyrimidine tracts, but further mapping experiments are
necessary for the exact localization.

S1 hypersensitive sites in human rDNA fragments

We wished to determine whether S1 sensitive DNA sequences
were also present in supercoiled plasmids of human rDNA. The
pE2 human clone contains the transcription initiation site, the
whole ETS and 1.7 kb of the 18S RNA gene. There was no
difference between the samples with and without S1 treatment
in the pE2 clone (Fig.3A), indicating that SHSs neither in the
ETS nor in the promoter region were present in this human
clone.

Results obtained in the pE6 plasmid, that contains the 3'
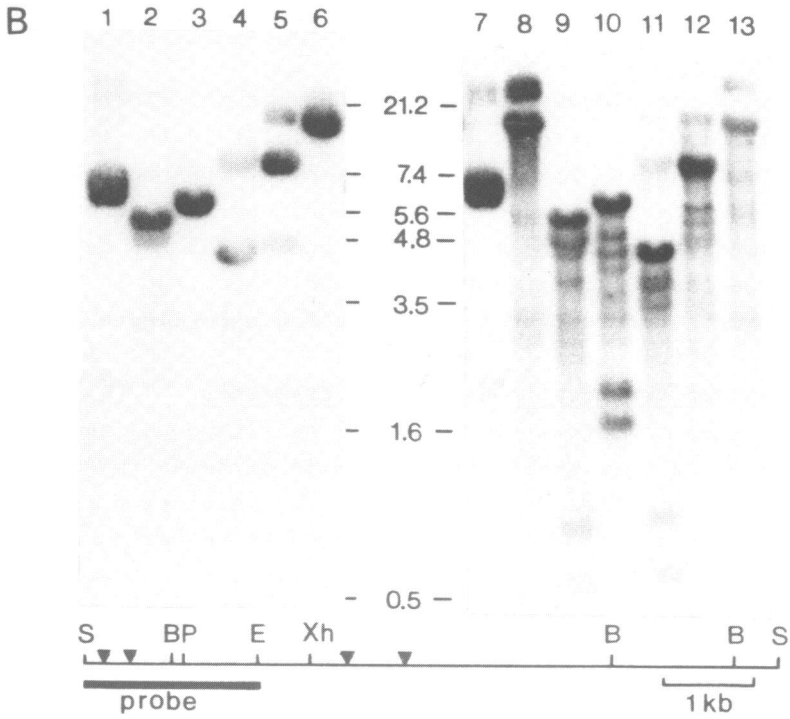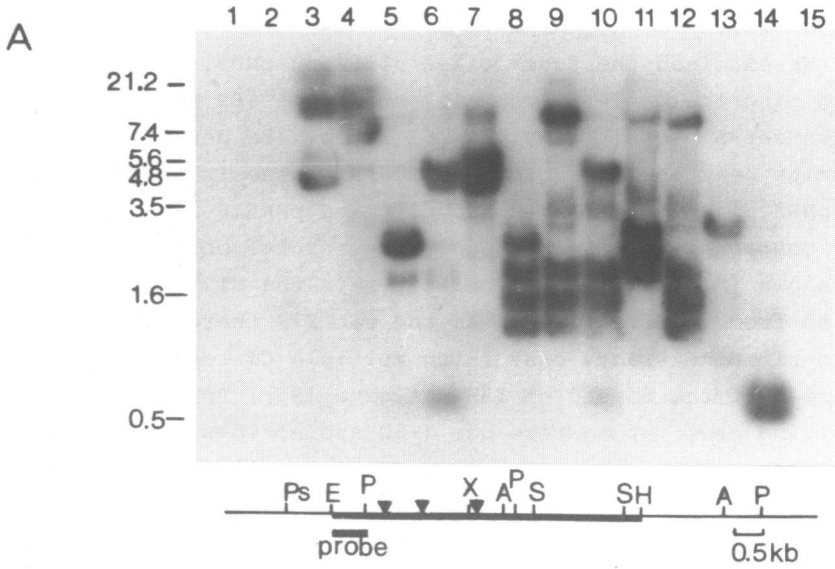
end of 18S RNA gene, both ITS 1 and 2, the 5.8S RNA gene and
about 3.6 kb of the 28S RNA gene are shown in Fig.3B. Two SHSs
were detected in this plasmid both in the 28S RNA coding region.
There were no SHS in the internal transcribed spacers.

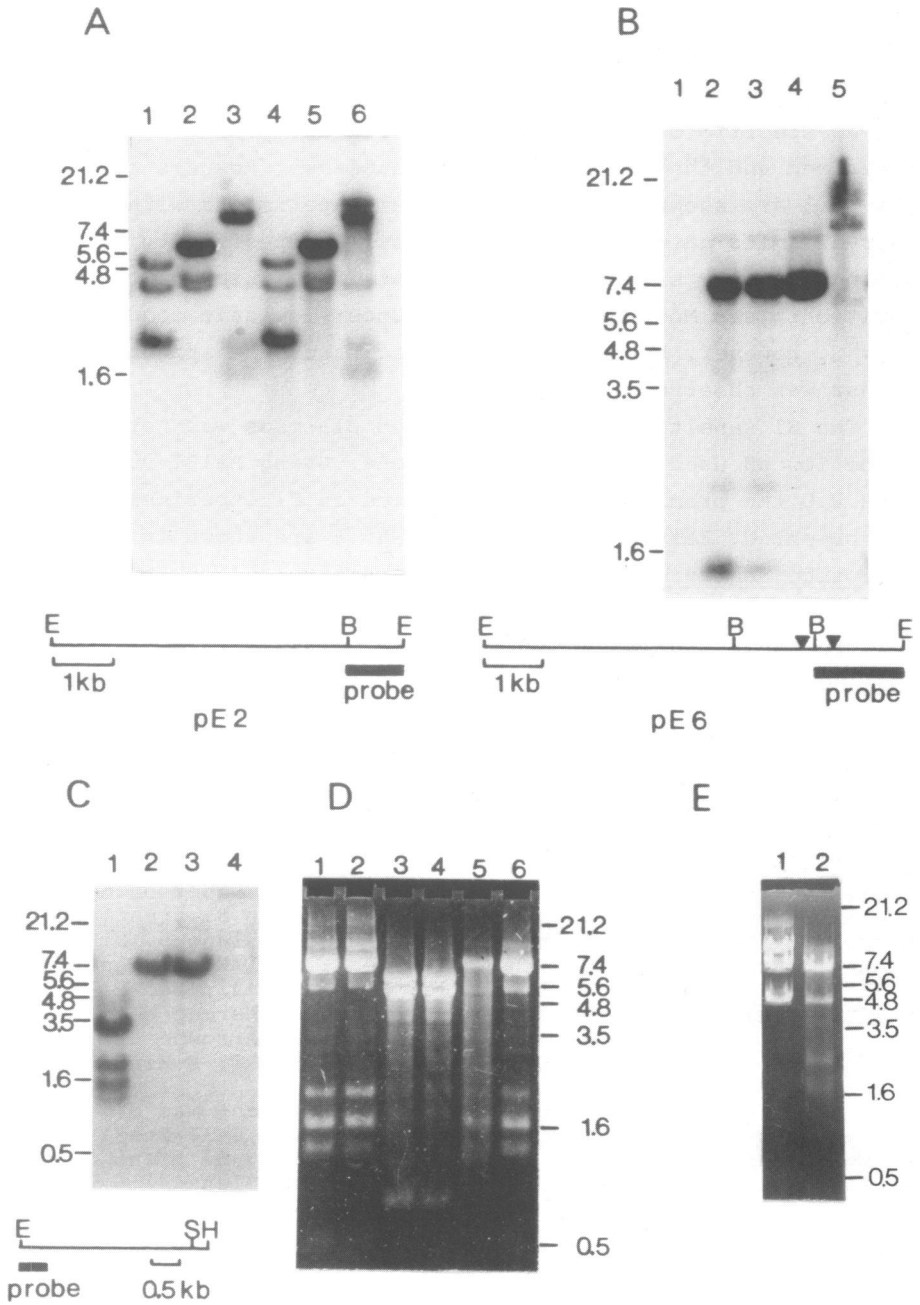Superhelicity requirement, salt and pH effects on Sl sensitivity

No specific Sl cleavage occurred if the plasmids were first
linearized and then treated with Sl nuclease. Results obtained
with p3.3 are shown in Fig.3C. Similar experiments with the
other plasmids gave identical results. The specificity of Sl
cutting was only slightly decreased at 25°C if low salt
conditions (see Methods) were used. Under low salt conditions
at 37°C, however, besides the specific bands the appearance of
a smear was observed (Fig.3D).

The Sl sensitivity of supercoiled plasmids was not caused
by the low pH used in the Sl digestions. Using Bal31 nuclease
at pH 8.0 the prominent bands, detected in Sl experiments were
also observed. Results obtained in pNH5.6 are shown in Fig.3E.

Nucleotide sequence of the Sl hypersensitive region in the rat
NTS

The nucleotide sequence of the EcoRI-SalI fragment (the 3'
end of the rat NTS) is shown in Fig.4. A 110 bp long fragment

Figure 2. Localization of major SHS in rat rDNA clones
A. Supercoiled p4.8 plasmid was Sl treated as described in
Methods and then digested with restriction enzymes. The resulted
fragments were probed with the end labeling EcoRI/PvuII fragment.
In this and in the subsequent experiments the molecular weight
markers (lanes 1, 2 and 15) were λDNA EcoRI and pBR322 HinfI
fragments, the lenghts are given in kilobases. Supercoiled p4.8
lane 3; Sl treated p4.8 lane 4. Plasmid without Sl treatment,
digested with AvaI/EcoRI lane 5; EcoRI/HindIII lane 6. Sl
treated plasmid digested with AvaI lane 7; AvaI/EcoRI lane 8;
EcoRI lane 9; EcoRI/HindIII lane 10; PstI lane 11; EcoRI/PstI
lane 12; PvuII lane 13; EcoRI/PvuII lane 14. The map below shows
the relevant restriction sites and the probe. Arrowheads
indicate the Sl sites. Symbols: A-AvaI; E-EcoRI; H-HindIII;
Ps-PstI; P-PvuII; S-SalI; X-XbaI.
B. Supercoiled pNH8 with and without Sl treatment was digested
with the enzymes indicated and probed with the SalI/EcoRI
fragment. Supercoiled pNH8 lane 1; pNH8 without Sl treatment
digested with BamHI lane 2; EcoRI lane 3; PstI lane 4; SalI
lane 5; XhoI lane 6. Supercoiled pNH8 lane 7; Sl treated pNH8
lane 8; Sl treated pNH8 digested with BamHI lane 9; EcoRI
lane 10; PstI lane 11; SalI lane 12; XhoI lane 13. In the map
below: B-BamHI; E-EcoRI; P-PstI; S-SalI; Xh-XhoI. Arrowheads
indicate the Sl sites.

in this region, starting from the EcoRI site, has recently been
sequenced (27).

Hybridization experiments suggested (28) the presence of
type2 Alu repeats in the NTS of the rat rDNA. In the sequenced
region two type2 Alu repeats and an ID sequence were found
(underlined in Fig.4). The ID sequence is believed to play a
specific role in the transcription or processing of brain-
-specific mRNAs (29). The significance of the ID sequence and the
Alu repeats in the 3' end of the rat NTS and also in the 5' end
of NTS (30) is not known at present. The most striking feature
of the sequenced region is the presence of a number of simple
sequences. There are $(TAAG)_7$ repeats at the 5' end and $(TAAG)_8$
and $(TAAA)_8$ repeats at the 3' end of the ID sequence. A $GT_{23}$
cluster (between nucleotides 681 and 726) a $GA_{15}$ repeat (between
nucleotides 1215 and 1244) and two long polypyrimidine tracts
(between 1491 and 1597 and between 1961 and 2052 nucleotides,
respectively) were found in the sequenced NTS fragment. In the
polypyrimidine tracts there are multiple CT and CCCT repeats
that were shown to be S1 sensitive in supercoiled plasmids in a
number of cases (6 and references therein). If the S1 treated
p4.8 plasmid was digested with XbaI enzyme,(the cleavage site

Figure 3A.,B. S1 hypersensitivity of human rDNA clones
A. The pE2 plasmid without S1 treatment digested with BamHI
lane 1; EcoRI lane 2; HindIII lane 3. S1 treated pE2 digested
with  BamHI lane 4; EcoRI lane 5; HindIII lane 6.
B. The pE6 plasmid was S1 treated in low salt (lane 2) or in
high salt (lane 3) conditions and then digested with EcoRI.
Plasmid without S1 treatment, digested with EcoRI lane 4;
S1 treated pE6 lane 5. Lane 1, markers. Map below: E-EcoRI;
B-BamHI. The arrowheads indicate the S1 sites.
    3C.,D.,E. Superhelicity requirement salt,and pH effects on
S1 hypersensitivity
C. S1 treated p3.3 digested with EcoRI/SalI lane 1; p3.3
linearized with EcoRI lane 2; p3.3 digested with EcoRI then
S1 treated lane 3. Markers lane 4.
D. Supercoiled p4.8, S1 treated in 300 mM NaCl at 37°C lane 1;
at 25°C lane 2; samples 3 and 4 are part of another experiment.
S1 treatment in 30 mM NaCl at 37°C lane 5, or at 25°C lane 6. In
low salt the specificity of S1 cutting decreased.
E. Supercoiled pNH5.6 was digested with Bal31 nuclease at pH 8.0
and then with EcoRI/HindIII (lane 2). Plasmid without Bal31
treatment, digested with EcoRI/HindIII (lane 1). In the Bal31
digested sample, 1.7 and 2.1 kb bands are seen (as in S1
treated pNH8).

```
      EcoRI                                                                                            ID
  1   GAATTCAAGG ACGAACGATT GTTTAAGTAT GACAAAAATA AGTAAGTAAG TAAGTAAGTA AGTAAGGGGG TTGGGGATTT AGCTCAGTGG TAGAGCGCTT

101   GCCTAGCAAG TGCAAGGCCT TGGGTTCTGT CCCCAGCTCC GAAAAAAAAG AAAGAAAAAA AAGTAAGTAA GTAAGTAAGT AAGTAAGTAA GTAAGTAAAT

201   AAATAAATAA ATAAATAAAT AAATAAATAC GATCATTTAG AAGTAATATA GGCAGAAGGA GAACATGTGG CTGAGGTGGT CTGAGGCTTC TGGCTGCTTT

301   TGGGTGCCTC TTCACCTTTC TGCACGTGCA AACTGAGTTT AATTGAGTTC TGTTTCCCTA GAGGCTGGGA AAGGGAGAAA CAGAAGTGAC CTGAACATCA

401   GGGCTAATGG CCGGCTCCTC CATCTTGGTG TCAGCCAAGC TGGCTGCCAA CCATCCACAG TTGAATCCAA AGTGCCCAAA TGGGACACTA TAACAAATCA

501   TGTATACAGA TTTGTGTATA TAGAAAACAT TTTTCCTTTC TCTTCTTTTC TTTTCTTGAG AAATTCAGCT GGTCCTGATG TCTTTCCCTC GGTGTGTCCC

601   TCTGTCATCT CTCTCTCTCT GTCTCTTTGT CTTACTGTCT CTGTCTGTCT CTGTCTGTCT CTGTCTCTGT CTCTCTCTCT GTGTGTGTGT GTGTGTGTGT
                                                                              Alu
701   GTGTGTGTGT GTGTGTGTGT GTGTGTTCCT TAATCTCAGC AGCACTACTC TATCCAATCG ATTTTTCCTC TCCCCTTGTC TCCTTCACAT GGCTATTACC

801   CCACCTTAAA CAGCACTAGA AGAGCCTGGC AGGCAGATAT CTACCCTCTA TGCTGTCAGA ATTTTTTTTT TTTTTTTTTA AAGTCAAAAC CTGATTCTCA

901   AGGAGCCATG TGGTGTGTCC TCCTAGGTTC TGGTGCCCTC TTCTGGCATG CAGACATATA GTCAGCAGAG AGAGCTGTGT GGGTGGCCCA GGCAGGCAGA

1001  GTGGGACCTCT GTGAGCTTGA GGCTAGATAA GTCTACGGAG CAAGTTCCAT GACTGCTGGG CTATACCAAA AGAAACGTAC CTTGAGGCAA AACAAAACAA

1101  AGCAGAAAAG GTTCTGCTTT TATTTTACAG ACATGTGTTC TCTCTAGTCC TATTGACTAT AGAAGTTTCA TCATGAAGTC ACATTGATTT TTATTACAGA

1201  GTTTATTTGT GTGTGAGAGA GAGAGAGAGA GAGAGAGAGA GAGAGTTTTT TCTTTAATCT TCAAACATAT TTTTTATTGT TTATTTATTT TATGAGCACA
                                                                      Alu
1301  CTGTCACTGT CTTCAACACA CAAGGAGAGG ATTCCATTAC AGATGGTTGT GAGCCACCAT GTGGTTGCTT GGATTTGAAC TGAGGATCTC TGGAAGAGCA

1401  GTCAGTGCTC TTCACCGCTG AGCCGTCTCT CCAGCCCTGA AACAATATCT TGAATAAACA ATAACAAAAA CACCAAAACT AAGTCTGCCA GTTTTCCTCT

1501  TTCTTTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCTCCCT CCCTCCCTCC CTCCCTTCCT TCTTTCTTCC TTTCTTCTCT TTTCTTCAGA

1601  TAGGATTTCT TAGTGAATGA TATTTTATGT TGATATAAAT TTTTTACGTA CTTTTGACTG GCTAACTAAC TAGCTAAGTC TGCCTGTCCT AAAGAAGGAG

1701  TTCCCCTGCC ACAACTAAAT ACGTGCATAG AAAAAAAATA TATAATTATT AACTTGGGAT GAAACTAATG TAACAAGCAA GCAAACAAAC GCACAAGCAA

1801  ACAAAGTAGG GATGGTGGTG CTGGGTGCCT GGTATATGGG AGATGGGAGA CCTGTGGTCT AGAGCGTCCA GAGCTCTGGC CTCCATACTC TGGACTGCAT

1901  TCTCTCTAGC AATCTTCCCA AAGTGGCTGT ATTTAACCAT GTTTTTATTT TTTAAATTGG CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT CTCTCTCTCT

2001  CTCTCTTTCT CCCTCCCTCC CTCCCTCCCT CCCTCCCTCC CTCCCTCCCC CCACCACACA CACAGAGGCA CGTGTGAACA AGGGAATGCA AATGCACAAA

2101  ATAAGTATGT TTTGATCAGA GTGAAAACGA ATCACGGGAA TTTTAACCAT TCCACCATCA TTCCACCATT CTTTTAATTT ACTCTCTGTG TTTAGTCTTC

2201  CAAAGAGGGT GGCAGGTGTG TGGTTTGGTG AGTGCCGGCT GGCGGTGGCA GCGAGGAACA GAGAACAACA ACAACCCCCC CTCCCCCCCA TCGGGATCGG

2301  TGGAGGGTTT TAAAAATGAG TGAGCCATTT AAAAATGGTG TGGAACTCAG AGGTCGCCGC CACCCTCCCC TCACAGACGG AGTTTAGGAC GCTCCCTTTT

2401  CCTAACTGTG CCTGTTCCTT GCTAATTGTT AACCGATTCG AGAGTGCATG TCCTTTCACT TAGAGGTGGC CTGTCCTAC CGCTCCGTCC TACTTTCCTT

2501  TTCTGGGTGC CTGAGGGGTT GGCCCCGAGT CGCCATCCCG CGTTCACTGT GTTCCGGCGG GACAACCGGG ACCGTTTGTG CCATTCGGGA GAAGTGGTGG

2601  GTACCTGCCC CGTTGTGCGT GGGTGAGTGT CCCCCTGAGC TAGGGAGGTC ACTCCGGAGA GACGGAATGA GTGTGTGTGG CGGCGGCGGC GCGTGACAGT

2701  CTGTATTGGT TTGCTTGATC GAGATCGCTG TCGGGCGACA CCTAGTGGCG ACAAGTTTCC GCAACGCTCC TGTCCCCCGG TTGGTGACAA AGGACGGGGA

2801  AGTGCCTGTG GTGAGCCGGC CAGGTGAGAG GGTGACAGGC AAGCAGGCGG GTGCGCGACG GAGACGCCGT CCTGTCTGGG GACCATCTCT AGCTAGGAGG
                                                                                                  Sal I
2901  TTGTACCTGA GAACTTCAGG GGAGGAGGGT GGCTGTACGA CCTATTGGGA CACAAGGAGG GGTTGGTGGC AGCTGGATGA GGTCGAC
```

## Figure 4. Nucleotide sequence of the rat NTS EcoRI/SalI fragment (p4.8 plasmid)

The type2 Alu repeats and the ID sequence are underlined and the arrowheads indicate the direction of possible transcription. Simple repeat sequences are shown by dashed lines. Since the exact number of nucleotides of the variable region (between the transcription initiation site and the SalI site) is not known, the sequence is numbered from the EcoRI site. The map position of the sequenced NTS region is shown in Fig.1.

is localized between the polypyrimidine tracts at nucleotide 1858) two broad bands,120-150 and 280-330 bp long respectively were observed (Fig.5A), indicating that S1 cutting occurred in the long polypyrimidine tracts.

## DISCUSSION

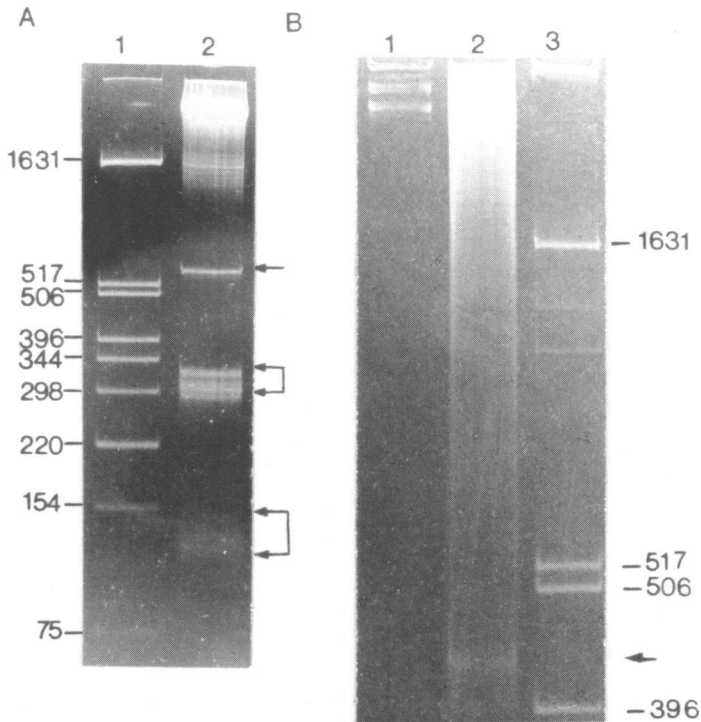The SHSs found in rat and human rDNA fragments in super-coiled plasmids are summarized in Fig.6. Sites 1 to 7 in the

Figure 5. Localization of SHSs in polypyrimidine tracts
A. S1 treated p4.8 plasmid was digested with XbaI,and the
resulted fragments were electrophoresed in 5% polyacrylamide
gel,lane 2. Marker: pBR322 HinfI fragments, lane 1. The broad
bands 120-150 and 280-340 nucleotides long respectively are the
results of S1 cutting in the 92 and 106 bp polypyrimidine.
polypurine tracts in the NTS (sites 2 and 3 in Fig.6).
B. The supercoiled pNH8 plasmid without (lane 1) and with (lane 2)
S1 treatment, was digested with SalI enzyme. The SalI digestion
generated  430 bp long fragments,indicating that S1 cutting
occurred in the polypyrimidine cluster at the 5' end of 18S RNA
gene (site 5 in Fig.6).

rat, and sites 1 and 2 in the human gene were determined

experimentally. The presence of SHSs downstream of the 28S RNA

gene are predicted in both species (site 8 in rat and site 3 in

the human rDNA in Fig.6). There are long (100 bp or longer)

polypyrimidine.polypurine tracts, containing multiple CT and

CCCT repeats in the sequenced 5' end regions of the two

mammalian NTSs (30, 31). Since a $(CT)_{12}$ repeat is sufficient to

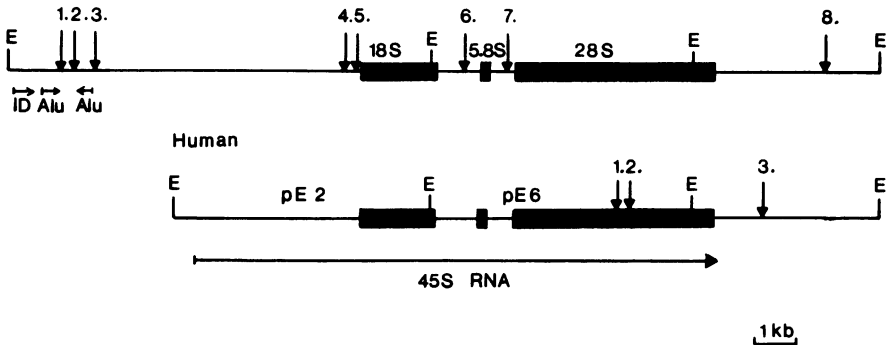generate SHS in pBR322 plasmid,we may predict that the rDNA NTS

Rat



Figure 6. Summary map of the SHSs in rat and human rDNA
In the transcribed part of genes (45S RNA) both the number and
distribution of SHSs are different in the two species. The SHSs
downstream of the 28S RNA gene appear to be conserved in the
two mammalian rDNAs. Further details are in DISCUSSION.

sequences, containing long CT elusters would be also S1 sensitive
in supercoiled plasmids.

The promoter regions of neither the rat nor of the human
rDNAs were S1 sensitive. Similar results were obtained in
Drosophila rDNA where the promoter region showed only weak S1
sensitivity (18). These results are in contrast with the S1
hypersensitivity of promoter regions of genes, transcribed by
polymerase II, and may reflect the differences in transcription
requirements of polymerases I and II. It is possible, however,
that S1 hypersensitive sites in the mammalian rDNA promoters can
only be detected in chromatin and not in supercoiled plasmids.

Sites 1 and 2 in the human 28S RNA gene may simply reflect
the foldback capability of the rRNA (18). Since sequence
differences exist even among the well conserved 28S RNAs (32)
the lack of SHS in the rat 28S RNA gene is not surprising. The
difference in S1 sensitivity of the transcribed spacers (ETS
and ITSs) may also be the result of sequence divergence. In the
rat rDNA there are long polypyrimidine.polypurine sequences
immediately upstream of the 18S and 28S RNA genes (25, 26), that
may have a role in the processing of the rRNAs (25). Site 5

certainly and site 7 probably are localized in these tracts. In the human gene the ETS and ITS are not S1 sensitive.

SHSs in the NTS, dowstream of the 28S RNA gene are apparently conserved in the rat and human rDNAs. The conserved SHSs found in similar position in three Drosophila species, were localized in AT rich cruciform structures (18). Sequence divergence of S1 sites is not unexpected, since the S1 enzyme can recognize, and cut, many different features in DNA (8, 13, 33, 34, 35). Further studies with the rDNA of different species will reveal if conserved SHS in the 5' end of the NTS are present in all eukaryotes. The conservation of SHSs in the rDNA NTS may or may not be correlated with the conservation of a specific function.

One may only speculate regarding the possible function of the S1 hypersensitive NTS regions of mammalian rDNAs. In the sequenced rat NTS fragment, besides the S1 sensitive (simple) sequences,a number of other simple sequences are also present. Simple sequences were suggested to have a role in the regulation of rDNA transcription, by influencing the positioning of nucleosomes in rDNA (27). This effect, however, is not specific for the rDNA chromatin,since simple sequences occur, on the average, once in every 6-8 kb DNA in the eukaryote genome (36).

Simple sequences are present not only in mammalian rDNA spacers but also in the spacers of the histone gene cluster in sea urchin (37, 38). They also occur between immunoglobulin genes (39, 40). There are a number of suggestions that different types of simple sequences are hot spots for recombination (41, 42, 43, 44). Interestingly these sequences are S1 sensitive (42, 44). It is tempting to speculate that simple sequences in the spacers of multigene families are involved in recombination processe (as hot spots). Simple sequences may allow localized exchange between members of multigene families, localized on homologous or on nonhomologous chromosomes (43) or may combine DNA segments that otherwise share no homology (45). It remains to be seen if recombination events, responsible for the maintenance of homogeneity within multigene families are

correlated with the presence of (Sl sensitive) simple sequences in the multigene family spacers.

At present there are a number of open questions regarding the exact role of SHSs and simple sequences in rDNA. Some of the questions may be better answered if data concerning the Sl and/or DNAse I hypersensitivity of mammalian rDNA chromatin will be available. Preliminary results,obtained in isolated nuclei, indicate that SHSs described in the present work are not correlated with gene activity.

REFERENCES
1. Elgin, S.C.R. (1981) Cell 27, 413-415.
2. Pederson, D.S., Shupe, K. and Gorovsky, M.A. (1984) Nucl. Acids Res. 12, 8489-8507.
3. Larsen, A. and Weintraub, H. (1982) Cell 29, 609-622.
4. Han, S., Udvardy, A. and Schedl, P. (1984) J. Mol. Biol. 179, 469-496.
5. Sheffery, M., Marks, P.A. and Rifhind, R.A. (1984) J. Mol. Biol. 172, 417-436.
6. Margot, J.B. and Hardison, R.C. (1985) J. Mol. Biol. 184, 195-210.
7. Weintraub, H. (1983) Cell 32, 1191-1203.
8. Cantor, C.R. and Efstratiadis, A. (1984) Nucl. Acids Res. 12, 8059-8072.
9. Emerson, B.M., Lewis, C.D. and Felsenfeld, G. (1985) Cell 41, 21-30.
10. Bonven, B.J., Gocke, E. and Westergaard, B. (1985) Cell 41, 541-551.
11. Luchnik, A.N., Bakayev, V.V., Yugai, A.A., Zbarsky, I.B. and Georgiev, G.P. (1985) Nucl. Acids Res. 13, 1135-1149.
12. Wu, C. (1980) Nature 297, 289-295.
13. Hentschel, C.C. (1982) Nature 295, 714-716.
14. Dybvig, K., Clark, C.D., Aliperti, G. and Schlesinger, M.J. (1983) Nucl. Acids Res. 11, 8495-8508.
15. LaVolpe, A.L., Taggart, M., McStay, B. and Bird, A. (1983) Nucl. Acids Res. 11, 5361-5380.
16. Bonven, B. and Westergaard, O. (1982) Nucl. Acids Res. 10, 7593-7608.
17. Palen, T.E. and Czech, T.R. (1984) Cell 36, 933-942.
18. Franz, G., Tautz, D. and Dover, G.A. (1985) J. Mol. Biol. 183, 519-527.

19. Birnboim, H.C. (1983) in Methods in Enzymology, eds. Wu, R., Grossman, L., Moldave, K. (Academic Press, N.Y.) Vol. 100, pp 243-255.
20. Rigby, P., Rhodes, D., Dieckman, M. and Berg, P. (1977) J. Mol. Biol. 113, 237-251.
21. Maxam, A.M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560-564.
22. Financsek, I., Mizumoto, K. and Muramatsu, M. (1982) Gene 18, 115-122.
23. Financsek, I. and Hidvégi, E.J. (1984) Acta Biochem. Biophys. Acad. Sci. Hung. 19, 185-192.
24. Wilson, G.N., Hollar, B.A., Waterson, J.R. and Schmickel, R.D. (1978) Proc. Natl. Acad. Sci. USA 75, 5367-5371.
25. Cassidy, B.G., Subramanyam, C.S. and Rothblum, L.I. (1982) Biochem. Biophys. Res. Comm. 107, 1571-1576.
26. Subramanyam, C.S., Cassidy, B., Busch, H. and Rothblum, L.I. (1982) Nucl. Acids Res. 10, 3667-3680.
27. Braga, E.A., Avdonina, T.A., Zhurkin, V.B. and Nosikov, V.V. (1985) Gene 36, 249-262.
28. Chikaraishi, D.M., Buchanan, L., Danna, K.J. and Harrington, C.A. (1983) Nucl. Acids Res. 11, 6437-6452.
29. Sutcliffe, J.G., Milner, R.J., Gottesfeld, J.M. and Lerner, R.A. (1984) Nature 308, 237-241.
30. Yang-Yen, H.F., Subramanyam, C.S., Cassidy, B. and Rothblum, L.I. (1985) J. Mol. Biol. 184, 389-398.
31. LaVolpe, A., Simeone, A., D'Esposito, M., Scotto, L., Fidanza, V., deFalco, A. and Boncinelli, E. (1985) J. Mol. Biol. 183, 213-223.
32. Gerbi, S.A., Gourse, R.L. and Clark, C.K. in The Cell Nucleus, eds. Busch, H. and Rothblum, L.I. (Academic Press, N.Y.) Vol. 10, pp 351-386.
33. Singleton, C.K., Klysik, J., Stirdivant, S.M. and Wells, R.D. (1982) Nature 299, 312-316.
34. Lilley, D.J. (1980) Proc. Natl. Acad. Sci. USA 77, 6468-6472.
35. Panayotatos, N. and Wells, R.D. (1981) Nature 289, 466-470.
36. Birnboim, H.C., Sederoff, R.R. and Paterson, M.C. (1979) Eur. J. Biochem. 98, 301-304.
37. Schaffner, W., Kunz, G., Daetwyler, H., Telford, J., Smith, H.O. and Birnstiel, M.L. (1978) Cell 14, 655-671.
38. Sures, I., Lowry, U.J. and Kedes, L.H. (1978) Cell 15, 1033-1044.
39. Richards, J.E., Gilliam, A.C., Shen, A., Tucker, P.W. and Blattner, F.R. (1983) Nature 306, 483-487.
40. Gebhardt, W. and Zachau, H.G. (1983) J. Mol. Biol. 170, 567-573.
41. Lilley, D.M.J. and Kemper, B. (1984) Cell 36, 413-422.
42. Kilpatrick, M.W., Klysik, J., Singleton, C.L., Zarling, D., Jovin, T.M., Hanan, L.H., Erlanger, B.F. and Wells, R.D. (1984) J. Biol. Chem. 259, 7268-7274.
43. Slightom, J.L., Blechl, A.E. and Smithies, O. (1980) Cell 21, 627-638.
44. Cohen, H.B., Effron, K., Rechavi, G., Ben-Neriah, Y., Zakut, R. and Givol, D. (1982) Nucl. Acids Res. 10, 3353-3370.
45. Tautz, D. and Renz, M. (1984) Nucl. Acids Res. 12, 4127-4137.