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New tandem repeat region in the non-transcribed spacer of human ribosomal RNA gene

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ABSTRACT

A new repetitive DNA region was identified in the non-transcribed spacer of human rDNA, namely a long (4.6 kb) sequence motif (XbaI element) was present in two copies. The repeating unit composed of two parts. One of them consisted of unique nucleotide sequences, interrupted by some simple sequences. The other, about 3.1 kb long one assembled only from highly repeated simple sequences. The unique sequence region contained two, inverted copies of the human AluI type repetitive DNA family. The authors suggest that the XbaI elements may flank the tandem arrays of human rRNA genes as terminal repeats and they might function both as the origin of rDNA replication and/or site of homologous recombination.

INTRODUCTION

The eukaryotic rRNA genes are arranged in tandemly repeated arrays in the nucleolus. A repeating unit composed of a region, encoding for the primary rRNA transcript and of a DNA region which is assumed not to be transcribed (non-transcribed spacer, NTS, recently called in some paper intergenic spacer, IGS). The nucleotide sequence of the mature rRNA transcripts of various eukaryotes exhibited a remarkable evolutionary conservation, while the length and nucleotide sequence of the non-transcribed spacer of rDNA (NTS) showed high diversity in different species (reviewed in ref. 1). The transcribed part of eukaryotic rDNA has been well studied, while the structure and the function of the NTS remained relatively unknown, especially in mammals (reviewed in ref. 2 and 3).

Previously we have studied the initiation (4, 5) and the termination (6) processes of mammalian rDNA transcription. Our results (7) as well as other data (8, 9, 10) suggested that in some extent the NTS of mammalian rRNA genes may be transcribed, similarly as recently reported for lower eukaryotes (11, 12, 13). For this reason we decided to examine the NTS of human rDNA in details.

In the present paper we report the cloning of the entire human NTS. Well inside the NTS a long repetitive element (XbaI element, 4.6 kb) was found and partially sequenced. Based on the revealed structure the possible function(s) of the XbaI element is discussed.

EXPERIMENTAL PROCEDURES

Bacterial strains: HB101 (F′, hsdS20(rB−,mB−), recA13, ara-14, proA2, lacY1, galK2, rpsL20(Sm+), xyl-5, mtl-1, supE44, lambda−); DH1 (F′, recA1, endA1, gyr96, thi-1, hsdR17(rk−,mk+), supE44, relA1?, lambda−); DH5-alfa (F′, endA1, hsdR17(rk−,mk+), supE44, thi-1, recA1, gyrA96, 080dlacZ M15, lambda−).

Materials: Enzymes and reaction kits were bought either from GIBCO-BRL GmbH (Eggenstein, West Germany) or from Amersham Int. (Amersham, England) and used

according to the suppliers recommendations. ZETA-probe nylon membrane was purchased from BioRad Lab. GmbH (Vienna, Austria). The alfa-P32-dATP was supplied by the Institute of Isotopes, Hungarian Acad. Sci. (Budapest, Hungary). DNA computer analysis was made by Stadden-plus software package of Amersham Int.

DNA sequencing was performed using either the dideoxy method (14, 15), as recommended by Amersham Int. or the chemical modification method (16).

Labelling of DNAs: The 4.6 kb long PstI fragment (Fig.1B) was labelled with the nick translation kit and the 0.49 kb XbaI−HincII fragment (Fig. 1B) with the primer extension kit of Amersham Int.

Alkaline Southern blotting and DNA hybridization reactions: Genomic DNA, following the restriction enzyme digestions was analyzed by electrophoresis on 1% agarose gel and alkaline blotted to nylon membrane (17). Prehybridization of filters was performed in 4×SSC, 10×Denhardt's solution (18), 0.1% SDS, 1mg/ml denatured chicken erythrocyte DNA and 50mM phosphate buffer (pH 7.0) at 65° C for 20 h. Hybridization reactions were performed in 4×SSC, 5×Denhardt's solution, 0.1% SDS, 100 μg/ml chicken erythrocyte DNA, 20mM phosphate buffer (pH7.0) and 1 million cpm/ml labelled DNA at 65° C for 12 hrs. Following hybridization, the filters were washed twice in 2×SSC, 0.1% SDS at 65° C for 1h and twice in 0.2×SSC, 0.1% SDS at 65° C for 1h, then autoradiographed.

RESULTS

New repetitive element inside the NTS of human rRNA gene.

The total NTS region of human rDNA has been cloned. Part of the NTS was subcloned from a cosmid clone harboring nearly the entire human rRNA gene (6). The remaining part of the NTS was cloned at the HindIII site of pUC19, following HindIII digestion of Hela genomic DNA.

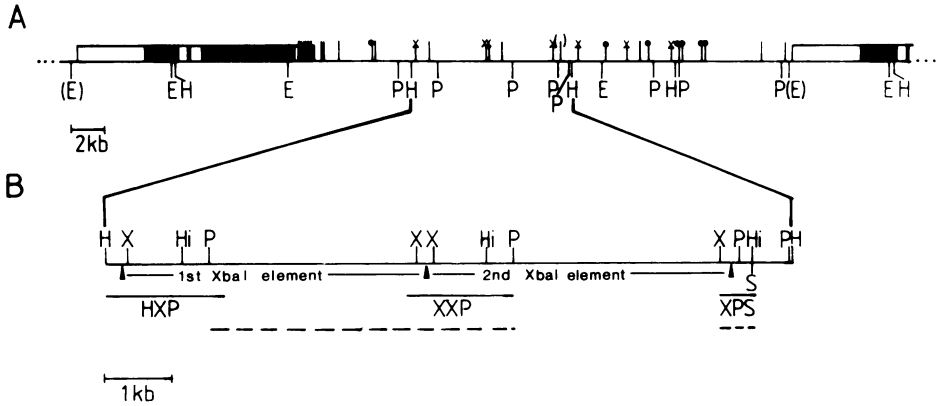


Figure 1. Structure of the human rRNA gene. A: Restriction map of the human rDNA. Detailed map is shown between the EcoRI site of 28S gene and the EcoRI site upstream of the transcription initiation point. Abbreviation of restriction sites: E, EcoRI; H, HindIII; P, PstI; |, HincII; ↑, BamHI and X, XbaI. Parenthesis indicates the partial presence of restriction enzyme recognition sites. B: Restriction map of the 10.3 kb HindIII fragment. Vertical arrows demonstrate the start and end points of the XbaI elements. Lines below the map, labelled with HXP, XXP and XPS, respectively indicate the sequenced regions. Dashed lines show the positions of DNA probes, used in hybridization reactions. H, HindIII; Hi, HincII; P, PstI; S, SalI and X, XbaI.

Detailed restriction maps of the coding region of rRNA gene were previously published (reviewed in ref. 19), but only the 5′ part of the NTS was mapped (20). We have constructed the restriction map of the entire NTS (Fig. 1A). The restriction map of the 5′ NTS corresponded to the formerly reported one (20). The restriction analysis revealed a new repetitive element within the NTS. A 4.3 kb long XbaI fragment (XbaI element) was present in two copies inside the 10.3 kb long HindIII fragment (Fig. 1B). The restriction analysis demonstrated an interesting feature of the XbaI element. No restriction recognition site has been found between the PstI site and the second XbaI cleavage site of the element. It represents an about 3.1 kb long simple sequence DNA region.

Noteworthy, the subcloning of the 10.3 kb long HindIII fragment was extremely difficult. The colonies, containing the entire fragment were retarded in growth and after a couple of days most of them consisted of a strongly deleted version of the cloned DNA. The deletion occurred only in replicating *E.coli.*

Sequence analysis of the XbaI element.

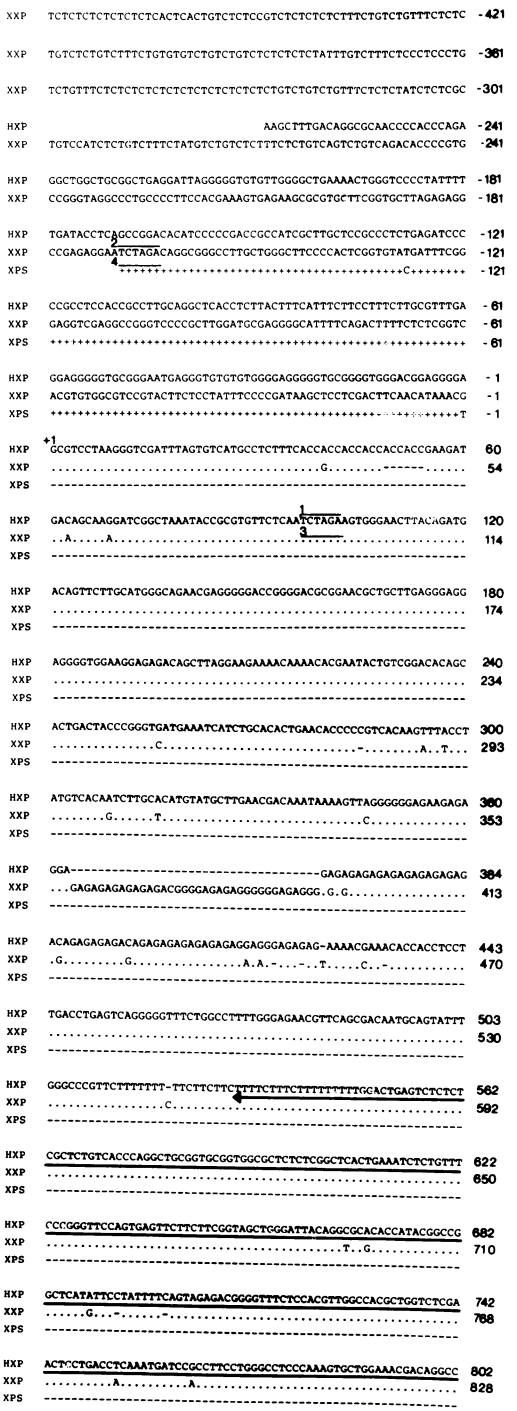
The nucleotide sequence of the HXP, XXP and XPS DNA regions, inside and flanking the XbaI elements (Fig. 1B) has been determined (Fig. 2). The sequence analysis revealed, that the first XbaI element started within the HXP region (Fig. 1B), 97 nt upstream of the first XbaI cleavage site of the 10.3 kb HindIII fragment (nt no.1 of HXP sequence in Fig. 2) and ended in the XXP region, 168 nt downstream of the second XbaI cutting site of the HindIII fragment (−2 nt of XXP in Fig. 2). The second element started 1 nt behind the first one and ended in the XPS region, 168 nt downstream of the fourth XbaI cleavage site of 10.3 kb HindIII fragment (−2 nt of XPS in Fig. 2). The nucleotide sequence of the two XbaI elements was found highly conserved (Fig. 2). The nucleotide sequence analysis showed a G-rich region upstream of the starting point of the first XbaI element (Fig. 2, from −60 to −1 nt of HXP sequence). The G-rich region was directly preceded by T-rich sequences. Downstream from the starting point of the XbaI elements a simple trinucleotide sequence (CAC) was repeated (from 36 nt to 53 nt in HXP). Further downstream inside the XbaI elements the simple GA motif was found 34 times in the first repeat (Fig. 2, HXP from 352 nt to 423 nt). Long T-stretches were present from the 511 nt to 547 nt in the first element (Fig. 2, HXP sequence) and in the corresponding part of the second one. Farther downstream a simple TAAC tetranucleotide was repeated several times.

The 3.1 kb long simple sequence region of the XbaI element started downstream of the PstI site and ended about 200 nt upstream of the second XbaI site of the repeat. The 5′ domain of this simple sequence region was very A-rich (Fig. 2, HXP sequence from 1371 nt). Further downstream an extremely long GC-rich region was detected (data not shown). The 3′ domain of the simple sequence region was pyrimidine rich and composed mainly of CT dinucleotides (Fig. 2, XXP sequence from −480 to −253 nt).

The analysis of the sequences, flanking 3′ of the second element revealed that an about 170 nt long region, surrounding the first and second PstI sites was present in a third copy (Fig. 2, XPS sequence from 21 to 190). Actually, it surrounded the third PstI site of the 10.3 kb HindIII fragment.

Type-I Alu repetitive sequences inside the XbaI element.

An AluI type repetitive sequence was found inside the XbaI elements, surrounding the PstI sites (Fig. 2, HXP and XXP sequences). These AluI repeats showed high homology to the consensus human AluI sequence (21), although an about 32 nt long region, which usually contains a homologous region to the viral replication origins (22) was deleted from



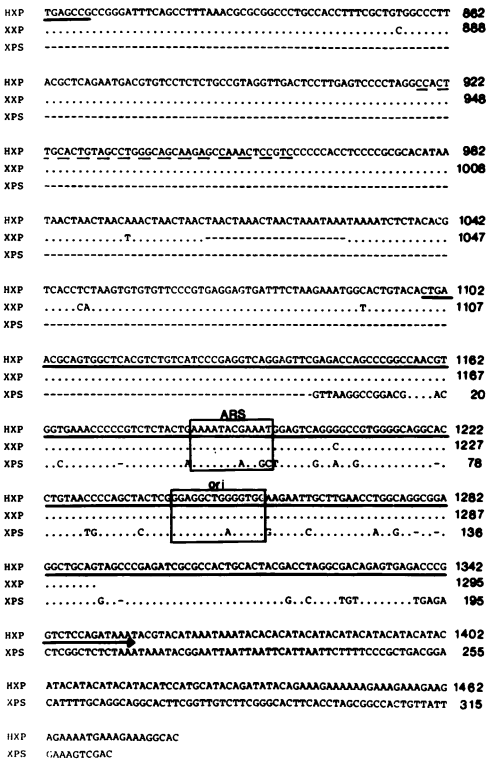


Figure 2. Comparison of nucleotide sequences of XbaI elements. The nucleotide sequence of the HXP, XXP and XPS regions (Fig. 1B) is shown. The numbering in HXP and XXP sequences starts at the beginning of the first and second XbaI element and continues downstream with positive numbers and upstream with negative numbers. The numbering of XPS sequence starts at the end of the second XbaI element and continues upstream with negative numbers and downstream with positive numbers. Space bars were introduced to maximize homology. Dots in XXP and XPS sequences represent the nucleotides homologous to HXP. The + mark in XPS show homology to XXP. AluI type sequences are underlined in HXP sequence and the arrowheads show the direction of Alu elements. A 42 nt long region homologous to the 3′ terminal sequence of two AluI repeats (23, 24) is demonstrated by dashed line below the HXP sequence. Sequences homologous to the replication origin of DNA viruses (PuGAGGCNGPuGGCGG, in ref. 22) and to the consensus of the autonomously replicating sequences of yeast (A/TAAAT/CATAAAA/T, in ref. 43) are boxed and labelled by ori and ARS, respectively. XbaI recognition sites are marked by line above the sequences and numbered according to their positions inside the 10.3 kb HindIII fragment (Fig. 1B).

the left arm (HXP in Fig.3). A third copy of the same AluI element was detected in the XPS region, directly downstream of the second XbaI element (Fig. 2 and XPS in Fig. 3). It surrounded the third PstI site of the 10.3 kb HindIII fragment.

An additional, more complete, but inverted copy of the AluI repetitive sequence family was also found within the XbaI elements (Fig. 2 and HXP.inv in Fig. 3). In this latter AluI member an about 10−14 nt long region was deleted from the right arm, comparing to the consensus human Alu sequence (Fig. 3). None of the AluI sequences were flanked by direct repeats. Interestingly, a 42 nt long region (Fig. 2, underlined by dashed line), positioned at about half way between the normally oriented and inverted Alu motifs showed 90% homology to the 3′ terminal sequence of two human Alu I sequences (23, 24).

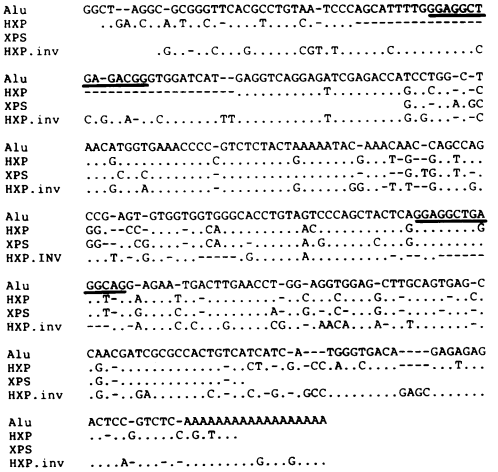


Figure 3. Nucleotide sequence comparison of different human AluI type repeats. Dots indicate the homologous nucleotides and space bars are introduced to maximize homology. Alu, a human AluI sequence (in ref. 21); HXP and HXP.inv, the Alu and the inverted Alu sequences of HXP (Fig. 2); XPS, the AluI repeat of XPS region (Fig. 2). Sequences, homologous to the viral replication origins (22) are underlined in Alu.

Genomic organization of the XbaI element.

To investigate the genomic organization of the XbaI element, HeLa genomic DNA was digested by various restriction enzymes, alkaline Southern blotted to nylon membrane (17) and hybridized with the labelled, longest (4.6 kb) PstI fragment of the 10.3 kb HindIII region (Fig. 4A). The 4.6 kb PstI fragment carried the entire XbaI element (Fig. 1B). The blotted genomic DNAs were also probed with the 0.49 kb XbaI−HincII fragment (Fig. 4B). This fragment carried the last 170 nt of the XbaI element, the AluI sequence and an additional, about 130 nt long downstream DNA region (Fig. 1B). The genomic DNA fragments, which contained only the AluI sequences of the XbaI element gave hardly visible bands with this probe because of the high number of interspersed Alu sequences throughout the genome. Hybridization to the HindIII digested HeLa genomic DNA gave a strong, 10.3 kb long band (Fig. 4A and B, lane 1), as it was expected from the restriction map of the cloned NTS. Positive signals, corresponding to the restriction map of the cloned DNA were also obtained by HincII (4.6, 4.0 and 3.4 kb), XbaI (4.3, 1.5 and 0.25 kb) and PstI (4.6, 3.5 and 2.2 kb) digestions (Fig. 4A, lanes 3, 6 and 8). In the HincII digested sample, however, the expected 4.0 kb band was rather faint and two additional, relatively strong 9.4 and 16 kb long bands were observed (Fig. 4A and B, lane 3). Latter bands disappeared at the HincII−HindIII double digestion, while the 4.6 kb band became more intense (Fig. 4A and B, lane 2). We interpreted this result, that the third HincII site of the HindIII fragment and the next, farther downstream occuring HincII site (Fig. 1A and B) were not present in all copies of the HeLa rDNA.

Beside the positive bands, which were either corresponded to the restriction map of the cloned DNA, or their presence may be explained by restriction fragment length polymorphism, some additional, fainter positive fragments were also observed in Fig. 4A. The presence of this faint bands may be partially explained by the occurrence of a minor length heterogeneity in the long simple sequence region of the XbaI element. The additional

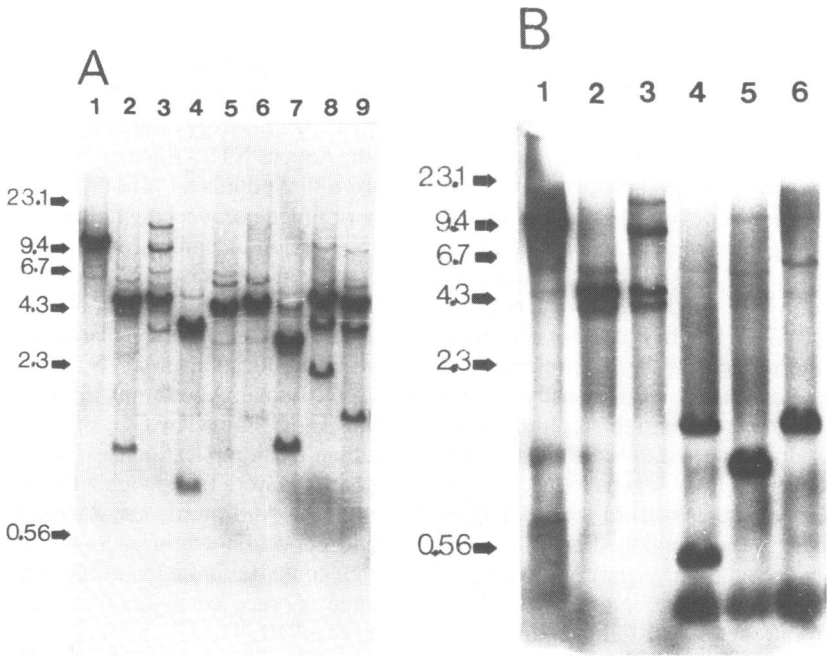


Figure 4. Genomic mapping with different regions of the 10.3 kb long HindIII fragment. A: HeLa genomic DNA was digested with restriction enzymes, alkaline blotted to nylon membrane and hybridized with the nick-translated 4.6 kb long PstI fragment (Fig. 1B), as described in Materials and Methods. B: The blotted genomic DNAs were hybridized with the 0.49 kb XbaI−HincII fragment (Fig. 1B), labelled by primer extension as described in Math. and Meth.. Lanes 1, HindIII; lanes 2, HincII−HindIII; lanes 3, HincII; lanes 4, HincII−XbaI; lanes 5, HindIII−XbaI; lanes 6, XbaI; lane 7, PstI−XbaI; lane 8, PstI and lane 9, PstI−HindIII. The arrows beside the autoradiogram indicate the positions of the 23.1, 9.4, 6.7, 4.3, 2.3 and 0.56 kb long lambda-DNA/HindIII markers.

positive fragments, such as the 7.1 and 6.1 kb long HindIII fragments (Fig. 4A, lane 1) probably represent homologous sequences elsewhere in the genome. Similar results were previously obtained by Kominami et al. in mouse, where a repetitive element of the NTS was found elsewhere in the genome, too (25, 26, 27, 28).

The genomic organization of the XbaI element was also investigated in human leukocyte genomic DNA (data not shown) and only one difference was detected, comparing it to the HeLa DNA. The third HincII site of the 10.3 kb long HindIII fragment was present almost in all copies of the leukocyte rDNA. The polymorphism between HeLa and leukocyte rDNAs probably represents differences among individuals (19).

DISCUSSION

The presence of repetitive DNAs is a general phenomenon of the non-transcribed spacer of rRNA genes. Various number of short tandemly repeated elements, as well as, highly repeated simple nucleotide sequences were described in the NTS of lower eukaryotes (29 and reviewed in ref. 2). The 5′ and 3′ terminal NTS of mouse, rat and human rRNA genes also contained short repetitive elements (30, 31, 32, 33, 34 and 35).

In this paper we identified a new repetitive element (XbaI element) in the non-transcribed

spacer of human rRNA gene. The XbaI element strongly differs from the previously reported subrepeats of the rDNA. The tandem subrepeats of the 5′ terminal spacer of mouse and rat rRNA genes are only 130−135 nt long (30, 31) and the repetitive unit of the human 3′ terminal NTS is 700−800 nt long (34). The copy number of these tandem units varies between and within individuals. Contrary to this, the XbaI element found by us is rather long (4.6 kb) and present only in two copies in the human NTS. Another feature of the XbaI element is its complexity. It is assembled from three domains. The 5′ domain (1.3 kb) and the 3′ domain (0.2 kb) composed mainly of unique sequences. Between the two unique sequence regions a very long (3.1 kb) DNA region assembled only from simple sequences. The 1.3 kb long 5′ domain contained two inverted representatives of the human AluI family. Alu and simple sequences are generally present in mammalian NTS (20, 33, 36, 37, 38, 39), but they have not been reported to be a common part of long tandem repeats.

Having sequenced the XbaI element and knowing the location in the NTS, one may speculate on the role of it. Usually the members of the human AluI family are containing a homologous region to the replication origin (PuGAGGCNGPuGGCGG) of some DNA viruses and suspected to serve as the origins of human DNA replication (reviewed in ref. 22). Sequences homologous to the viral replication origins were found within the normally oriented AluI sequences of the XbaI elements and in the AluI motif, just downstream of the tandem repeat region (Fig. 2). In the minimal replication origin of SV40 the 14 nt long conserved motif is part of a palindrome, which contains binding sites (GAGGC) for the large tumor antigene of SV40. This palindrome is preceded by another incomplete palindrome region and followed by AT-rich sequences (40, 41, 42). Similar palindrome structure was found within the XbaI element around the ori region of Fig. 2 (data not shown). Interestingly, just 5′ to this region a homology was found to the consensus sequence (A/TAAAT/CATAAAA/T) of the autonomously replicating sequences of yeast (reviewed in ref. 43) (ARS in Fig. 2).

Unequal crossing over and gene conversion has been suggested for the maintenance of the homogeneity of mammalian rRNA genes within a species (reviewed in ref. 44). Unequal exchange occurs between the newly replicated sister molecules and highly repeated DNA sequences, mainly AT-rich regions and GC-clusters provide a good opportunity for the binding of recombination enzymes. The proximity of the DNA replication origin to the recombination hot spots may increase the efficiency of recombination processes (reviewed in ref. 45). The XbaI element may contain a replication origin (see above) and about 100 nt downstream of this site a very long (3.1 kb) simple sequence DNA region starts. It begins with an AT-rich region and continues with GC-rich sequences. The DNA structure and the extreme unstability of this region in replicating *E.coli* may suggest a potential role for it in the recombination pathways.

Putting all of these together we propose that the recombinogenic unit of the human rRNA genes starts at the first nucleotide of the second XbaI element, continues through the rest of the non-transcribed spacer, then proceeds through the transcribed portion of rDNA, passes along the NTS sequences 3′ to the 28S rRNA gene and then finally ends at the last nucleotide of the first XbaI element. By this way the repeating arrays of the human rRNA gene are flanked at both sides by rather long (4.6 kb) terminal repeats, namely by the XbaI elements. These terminal repeats may function both as the origin of the replication of rDNA and as target sites for the homologous recombination of the rRNA genes.

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REFERENCES

1. Gerbi, S.A (1985) In McIntyre, R.J. (ed.) Molecular Evolutionary Genetics, Plenum Publishing Corp. pp. 419–517.
2. Moss, T., Mitchelson, K. and DeWinter, R.F.J. (1985) In Maclean N. (ed.) Oxford Suveys on Eukaryotic Genes 2, Oxford University Press, Oxford, pp. 207–250.
3. Sollner-Webb, B. and Tower, J.: (1986) In Richardson, C.L. (ed) Ann. Rev. Biochem., Annual Reviews Inc., Palo Alto, California, USA. 55, 801–838.
4. Financsek, I. and Hidvegi, E.J. (1984) Acta Biochim. et Biophis. Acad. Sci. Hungary, 19, 185–192.
5. Safrany, G., Tanaka, N., Kishimoto, T., Ishikawa, Y., Kato, H., Kominami, R. and Muramatsu, M. (1989) Mol. Cell. Biol. 9, 349–353.
6. Safrany, G., Kominami, R., Muramatsu, M. and Hidvegi, E.J. (1989) Gene, in press
7. Hidvegi, E.J., Prestayko, A.W. and Busch, H. (1971) Physiol. Chem. Phys. 3, 17–35.
8. Henderson, S. and Sollner-Webb, B. (1986) Cell 47, 891–900.
9. Grummt, I., Kuhn, A., Bartsch, I. and Rosenbauer, H. (1986) Cell 47, 901–911.
10. Baker, S.M. and Platt, T. (1986) Cell 47, 839–840.
11. Labhart, P. and Reeder, R.M. (1986) Cell. 45, 431–443.
12. De Winter, R.F.J. and Moss, T. (1986) Nucl. Acids Res. 14, 6041–6051.
13. Tautz, D. and Dover, G.A. (1986) EMBO J. 5, 1267–1273.
14. Sanger, F., Nicklen,S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
15. Messing, J. (1983) Meth. Enzymol. 101, 20–89.
16. Maxam, A.M. and Gilbert, W. (1980) Meth. Enzymol. 65, 499- 559.
17. Reed, K.C. and Mann, D.A. (1985) Nucl. Acids Res. 13, 7207- 7221.
18. Denhardt, D. (1968) Biochem. Biophys. Res. Commun. 23, 641- 652.
19. Wilson, G.N. (1982) In Busch, H. (ed.) The Cell Nucleus, Academic Press, New York, USA, 12. 287–318.
20. Higuchi, R., Stang, H.D., Browne, J.K., Martin, M.O., Huot, M., Lipeles, J. and Salser, W. (1981) Gene 15, 177–186.
21. Pan. J., Elder, J.T., Duncan, C.M. and Weissman, S.M. (1981) Nucl.Acids Res. 9, 1151–1170.
22. Doolitle, W.F. (1985) In Cavalier-Smith, T. (ed.) The Evolution of Genome Size, J.Wiley and Sons LTD, Chichester, pp. 443–487.
23. Bell, B.J., Pictet, R. and Rutter, W.J. (1980) Nucl. Acids Res. 8, 4091–4109.
24. Deininger, P.L., Jolly, D.J., Rubin, C.M., Friedman, T. and Schmied, C.W. (1981) J. Mol. Biol. 151, 17–33.
25. Kominami, R., Urano, Y., Mishima, Y., Muramatsu, M., Moriwaki, K. and Yoshikura, H. (1983) J.Mol.Biol. 165, 209- 228.
26. Kominami, R., Muramatsu, M. and Moriwaki, K. (1983) Nature 301, 87–89.
27. Kominami, R., Sudo, K., Yoshikura, H., Suzuki, H., Moriwaki, K., Hilgers, J. and Muramatsu, M. (1985) J.Mol.Biol. 183, 301–309.
28. Kominami, R. and Muramatsu, M. (1987) J.Mol.Biol. 193, 217- 222.
29. Tautz, D., Tautz, C., Webb, D. and Dover, G.A. (1987) J. Mol. Biol. 195. 525–542.
30. Arnheim, N. and Kuehn, M. (1979) J. Mol.Biol. 134, 743–765.
31. Chikaraishi, D.M., Buchanan, L., Danna, K.J. and Harrington, C.A. (1983) Nucl. Acids Res. 11, 6437–6452.
32. Grummt, I., Maier, U., Ohrlein, A., Hassouna, N. and Bachellerie, J.P. (1985) Cell 43, 801–810.
33. Yavachev, L.P., Georgiev, O.I., Braga, E.A., Avdonina, T.A., Bogomolova, A.E., Zhurkin, V.B., Nosikov, V.V. and Hadjiolov, A.A. (1986) Nucl. Acids Res. 14, 2799–2810.
34. La Volpe, A., Simeone, A., D'Esposito, M., Scotto, L., Fidanza, V., de Falco, A. and Boncinelli, E. (1985) J.Mol.Biol. 183, 213–223.
35. Krystal, A. and Arnheim, M. (1978) J.Mol.Biol. 126, 91–104.
36. Hsin-Fang Yang-Yen, Subrahmanyam, C.S., Cassidy, B. and Rothblum, L.I. (1985) J.Mol.Biol. 184, 389–398.
37. Financsek, I., Tora, L., Kelemen, G. and Hidvegi, E.J. (1986) Nucl.Acids Res. 14, 3263–3277.
38. Mroczka, D.L., Cassidy, B., Busch, H. and Rothblum, L.I. (1984) J.Mol.Biol. 174, 141–162.
39. Brownell, E., Krystal, M. and Arnheim, N. (1983) Mol. Biol. Evol. 1. 29–37.

40. Campbell, J.L. (1986) In Richardson, C.C. (ed.) Annual Review of Biochemistry, Annual Reviews Inc., Palo Alto, California, USA. 55, 733–771.
41. Dean, F.B., Borowiec, J.A., Ishima, Y., Deb, S., Tegtmeyer, P. and Hurwitz, J. (1987) Proc. Natl. Acad. Sci. USA 84, 8267–8271.
42. Borowiec, J.A. and Hurwitz, J. (1988) Proc. Natl. Akad. Sci. USA 85, 64–68.
43. Campbell, J. (1988) TIBS. 13, 212–217.
44. Treco, D., Brownell, E. and Arnheim, N. (1982) In Busch, H. (ed.) The Cell Nucleus, Academic Press, New York, USA, 12, 101–126.
45. Dyson, D. and Sherrat, D. (1985). In Cavalier-Smith, T. (ed.) The Evolution of Genome Size. J. Wiley and Sons. Chichester, pp. 353–395.

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