Volume 10 Number 24 1982 Nucleic Acids Research

A nonanucleotide sequence involved in promotion of ribosomal RNA synthesis and RNA priming of DNA replication in yeast mitochondria

K.A.Osinga, M.De Haan, T.Christianson* and H.F.Tabak

Section for Molecular Biology, Laboratory of Biochemistry, University of Amsterdam, Kruislaan 318, 1098 SM Amsterdam, The Netherlands and *Departments of Medicine, Biochemistry, Biology and Pathology, University of Chicago, Chicago, IL 60637, USA

Received 11 October 1982; Accepted 16 November 1982

ABSTRACT

We have examined the initiation of transcription of the mitochondrial genes for ribosomal RNA (rRNA) in the yeast Kluyveromyces lactis and show that these are transcribed independently from individual promoters. The mature large rRNA contains a 5' di- or triphosphate end which can be labelled in vitro with [α-^{32}P]GTP using guanylyltransferase and this enabled us to determine the nucleotide sequence of its 5' terminus. For the small rRNA, a minor in vitro capped RNA species hybridizes in the region where - as judged from S_1 nuclease protection experiments - the precursor of this RNA starts. We have determined the DNA sequence around the beginning of both rRNA genes and this reveals the existence of an identical nonanucleotide sequence (5' -ATATAAGTA- 3') just preceding the positions where the rRNAs start. This sequence is identical to the one preceding the rRNA genes in the mtDNA of the distantly related yeast Saccharomyces cerevisiae (Osinga, K.A. and Tabak, H.F. (1982) Nucl.Acids Res. 10, 3617-3626) and supports our proposal that this sequence motif is part of a yeast mitochondrial promoter.

We have noticed that the same sequence is located in the putative origin of replication present in hypersuppressive petite mutants of S. cerevisiae and consider the possibility that this sequence is involved in RNA priming of DNA replication.

INTRODUCTION

Yeast mtDNA codes for a limited set of proteins, 25 tRNAs and two rRNAs. The transcription of these genes has been studied in detail, but the information gathered has resulted primarily only in the localization of RNAs on the physical map and in partial elucidation of the intricate processes involved in RNA splicing [1,2]. Much less is known about initiation of transcription. However, separate promoters responsible for de novo initiation of transcription of rRNA genes exist. Transcripts of both genes can be labelled with [α-^{32}P]GTP and guanylyltransferase, which is evidence for the presence of a di- or triphosphate group at the 5'-end derived from initiation of transcription [3,4]. Both genes have identical nucleotide sequences

© IRL Press Limited, Oxford, England.

0305-1048/82/1024-7993$2.00/0

precisely at the positions where initiation of rRNA synthesis occurs and this identical nucleotide sequence may well be part of their promoter [5].

In order to assess further the significance of this common sequence motif for the initiation of transcription, we have studied the synthesis of mitochondrial rRNAs in Kluyveromyces lactis. This yeast is only distantly related to Saccharomyces cerevisiae: its mtDNA is only half the size of that in S. cerevisiae [6], the gene order is quite different [7-9], and DNA-DNA hybridization shows that both nuclear and mtDNAs of K. lactis gave only 10% cross-hybridization with their counterparts in S. cerevisiae [10]. Nevertheless, we have discovered that both rRNA genes are preceded by the same nonanucleotide sequence which is identical to that found by us in S. cerevisiae [5] and which occurs at the same position relative to the beginning of the genes. This sequence has obviously been conserved in evolution and this supports our proposal that it is part of a yeast mitochondrial promoter. We have also found this motif in the nucleotide sequences held in common by hypersuppressive petite mutants of S. cerevisiae, which have retained a putative origin of DNA replication and we discuss the possibility that the sequence is involved in RNA priming of DNA synthesis.

MATERIALS AND METHODS

The general procedures for growth of the wild-type yeasts S. cerevisiae and K. lactis (strain NRRL Y-1440), purification of mtDNA, mtRNA and recombinant plasmid DNA, restriction enzyme incubation, S_1 nuclease analysis, labelling DNA by nick-translation or T_4 polynucleotide kinase and Southern blot hybridization have been described previously [5,11]. For size calibration we used a 5'-terminally labelled HinfI digest of pBR322 (1631, 517, 506, 396, 344, 298, 221, 220, 154 and 75 bp).

Construction and screening of recombinants: K. lactis mtDNA was restricted with MboI and ligated into the BamHI site of pBR313 [12], previously treated with bacterial alkaline phosphatase. After 16 h ligation at 13°C, Escherichia coli strain HB490 was transfected and transformants selected on ampicillin-containing plates. Colony filter hybridization was performed according to Grunstein and Hogness [13] with heterologous probes from S. cerevisiae KL14-4A, containing the 5' borders of the large and small rRNA genes. In other experiments we ligated MboI-digested K. lactis mtDNA to phage M13 mp7 [14], cut with BamHI and transfected into E. coli strain JM103. DNA, isolated from white plaques, was screened with specific S. cerevisiae probes (see before). Plasmid DNA and the replicative form of the phages was isolated from positive recombinants to characterize the inserted fragments in more detail by restriction enzyme analysis and hybridization

with isolated 15S or 21S rRNA from KL14-4A. pBR313 clone Pl-15A contains a 2300-bp MboI insert with the 5'-end of the small (15S) rRNA gene, M13 clone M-20A has a 1100-bp insert containing the 5'-end of the large (20S) rRNA gene (see Figs 1 and 2, lanes b, e and g for characterization by hybridization). From these primary recombinant clones smaller sub-clones were constructed to aid the DNA sequence analysis. This was carried out as follows: Clone M-20A contains the 1100-bp K. lactis fragment in the vector M13 mp7. This vector is a derivative of the EcoRI vector M13 mp2 and contains in the single EcoRI site a short synthetic DNA fragment (42 bp) with recognition sites for several enzymes, suitable for cloning [14]. This fragment is a perfect inverted repeat and all sites occur twice in reverse order (e.g. EcoRI-MspI-BamHI-AccI----AccI-BamHI-MspI-EcoRI). Since the 1100-bp insert lacks an EcoRI site, EcoRI digestion of the replicative form of M-20A generates a yeast fragment, elongated at both sites with a short piece of M13 mp7 DNA (vide supra), containing an MspI site. This fragment was purified and restricted with MspI, which cuts only once in the yeast fragment. This results in two fragments with protruding ends which were ligated into M13 mp7, cut with AccI. After transfection into JM103, sub-clones M-20A1 and M-20A2 were isolated. M-20A1 contains the 5'-end of the large rRNA gene. The procedure for isolating sub-clones from Pl-15A was as follows: the yeast fragment containing the beginning of the small rRNA gene was taken out of clone Pl-15A by digestion with HhaI (see Fig. 1). This fragment was mixed with EcoRI vector M13 mp7 and protruding single-stranded ends were removed by incubating the fragments with 1 unit DNA polymerase I (Klenow fragment) in the presence of all four deoxynucleotides (75 μM each) for 30 min at room temperature in a final volume of 7.5 μl. The reaction was terminated by heat inactivation for 10 min at 65°C and yeast DNA fragment and vector were linked together by blunt-end ligation with 1 unit T_4 DNA ligase and 1 mM ATP. E. coli JM103 was transfected with this mixture and transformants isolated and screened as described before. In this ligation procedure, EcoRI restriction sites are regenerated.

DNA sequencing: Single-stranded template DNA for use in the dideoxy sequence method of Sanger et al. [15] was prepared from recombinant M13 plaques [16] and complementary DNA synthesis started with a 18-mer synthetic primer (a gift from Prof. J.Van Boom, Leiden), complementary to the sequence of M13 flanking the insert.

5'-End-labelling of primary transcripts and RNA sequencing: Primary transcripts of K. lactis mtRNA were 5' end-labelled in vitro using the capping enzyme guanylyltransferase from Vaccinia. The procedure followed was essentially the same as described by Levens et al. [3]. The RNA sequence was determined enzymatically as described [17]. Analysis of initiating nucleotides of mtRNA by nuclease P_1 digestion and thin-layer chromatography was performed as described [3].

Southern-blot hybridization with capped mtRNA was performed after treatment of the RNA with DNase I and alkali, according to Levens et al. [3], to prevent hybridization with restriction fragments not containing a transcription-initiation site.

RESULTS

Initiation of transcription of the rRNAs

In contrast to the situation in S. cerevisiae, the large (20S) and small (15S) mitochondrial rRNA genes of K. lactis are found close together on the mitochondrial genome [7,9] with an

intergenic distance of about 2.2 kb (Fig. 1). To study the mode of transcription we have first determined the direction of transcription of the two genes. By screening an MboI clone bank of K. lactis mtDNA with heterologous S. cerevisiae probes we have isolated recombinants containing the 5' border of each rRNA gene. These recombinant clones (Pl-15A and M-20A) were characterized by restriction endonuclease analysis and located on the physical map of K. lactis mtDNA as summarized in Fig. 1. From the primary recombinants smaller sub-clones were constructed (see Methods) as outlined in Fig. 1. The hybridization of M-20A and M-15A1 with a HindII+III digest of K. lactis mtDNA shows that fragments TD1 and TT8 contain the 5'-end of the large and small rRNA, respectively (Fig. 2, lanes c, f and i). This implies that both genes are transcribed from the same DNA strand in the order 5' - 15S - 20S - 3' (see Fig. 1).

We have used the guanylyltransferase capping assay to study whether the rRNA genes are transcribed separately. The most prominent in vitro capped transcript in total mtRNA preparations co-migrates with mature 20S rRNA (Fig. 3, lanes c and d).

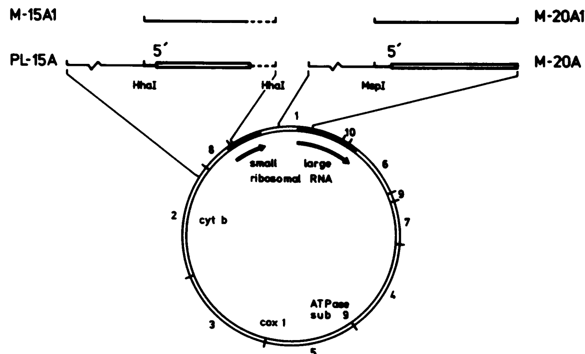

Fig. 1. The HindII and HindIII physical maps of K. lactis mtDNA and the localization of the two rRNA genes and the genes coding for cytochrome b, COX 1 and ATPase subunit 9 (from refs 7-9). Numbers indicate the order of HindII+III fragments according to the system developed by Sanders et al. [25]. Arrows indicate the direction of transcription of the rRNA genes (see text for explanation). Recombinant clones Pl-15A and M-20A contain MboI inserts originating from the 5' flank of the 15S and 20S rRNA genes, respectively. M-15A1 and M-20A1 are sub-clones (see Methods for construction). Dotted lines in M-15A1 represent the pBR313 sequence part sub-cloned from Pl-15A into M-15A1.

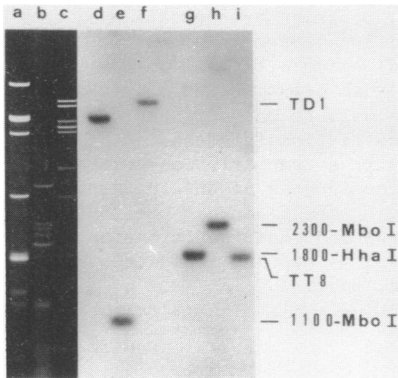

Fig. 2. Polarity of transcription of the rRNA genes. Digestions of K. lactis mtDNA were separated on a 0.8% agarose gel (lanes a-c), blotted onto nitrocellulose filters and hybridized with probes specific for the 5'-end of the large (lanes d-f, clone M-20A) or small (lanes g-i, clone M-15A1) rRNA gene. Lanes a, d and g, wild-type mtDNA x HhaI; b, e and h, wild-type mtDNA x MboI; c, f and i, wild-type mtDNA x HindII (T) x HindIII (D). Indications TD1 and TT8 refer to the wild-type HindII+III map of K. lactis mtDNA (see Fig. 1). Other relevant hybridizing fragments are indicated by their length.

The partial sequence analysis (vide infra) demonstrates that this labelled RNA is the large rRNA and we conclude that transcription of the large rRNA gene is initiated de novo.

The evidence supporting de novo transcription of the small rRNA gene is indirect. When in vitro capped mtRNA is used for hybridization with Southern blots of MboI and HhaI mtDNA fragments (Fig. 4), a primary transcript maps in the region where 15S precursor RNA starts according to S_1 nuclease analysis (see below). Identical Southern blots have also been hybridized with 5'-specific 20S and 15S rDNA probes (Fig. 2). Comparison of both experiments (Figs 2 and 4) shows that the fragments strongly hybridizing with the capped RNA correspond to those containing the 5'-end of the 20S rRNA gene, whereas the weakly-hybrid-

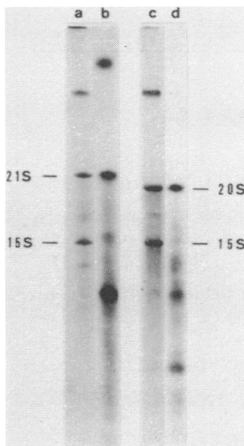

Fig. 3. Analysis of primary transcripts. Total mtRNA of S. cerevisiae (KL14-4A; lanes a and b) and K. lactis (lanes c and d) has been capped in vitro with guanylyltransferase and separated on a 1.5% agarose-6 M urea gel. Lanes a and c, ethidium bromide fluorescence; b and d, autoradiograms. The positions of mature rRNAs have been indicated.

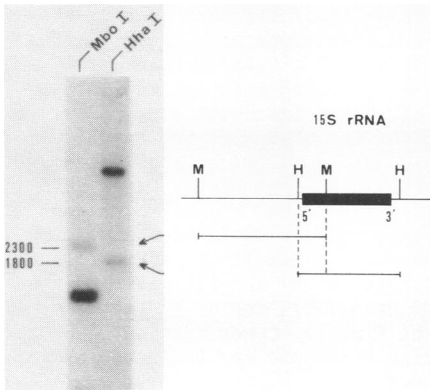

Fig. 4. Southern hybridization of total mtRNA capped in vitro to mtDNA digested with MboI or HhaI. The RNA has been treated with DNase I and alkali to prevent hybridization with restriction fragments not containing a transcription-initiation site. The lengths of the fragments containing the 5'-end of the 15S rRNA gene and their positions on the physical map are indicated. M, MboI; H, HhaI.

izing bands (Fig. 4, 2300 bp MboI and 1800 bp HhaI) contain the 5' border of the 15S rRNA gene (Fig. 2, lanes g and h). It is obvious that the primary transcript of the small rRNA gene is only a minor species in total mtRNA. The absence of an in vitro capped transcript at the position of 15S rRNA indicates a processing event at the 5'-end (Fig. 3, lanes c and d). All these results are in accordance with the existence of a precursor RNA that is processed at the 5'-end into mature small rRNA as we have shown for S. cerevisiae [4,11].

The results prove that in contrast to the situation in most other organisms, a common precursor for the rRNAs does not exist. It is, therefore, interesting to compare the DNA sequence flanking the rRNA genes to see if common sequence motifs are involved in the initiation of their transcription.

Determination of DNA sequences around the position at which the rRNA genes start

The position at which the 20S rRNA gene starts was established in two ways:

1. The isolated 1100-bp MboI insert from clone M-20A (see Fig. 1) was labelled at the 5'-end, hybridized with total mtRNA, treated with S_1 nuclease and the protected hybrids analysed by agarose gel electrophoresis (Fig. 5A). The labelled MboI fragment has also been digested with MspI (resulting in two fragments of 650 and 400 bp, respectively) to measure the distance between the MspI site and the 5'-end of the gene. The length of the S_1 nuclease-resistant hybrid (570 bp) indicates that the 5'

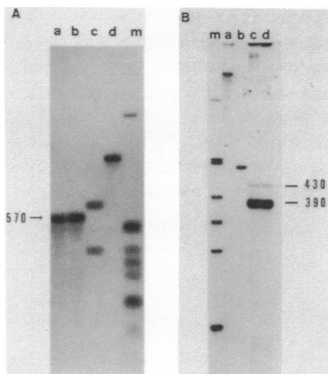

Fig. 5. Determination of the position of the 5'-ends of the rRNA genes with respect to the physical map by S_1 nuclease protection analysis. Hybrids were formed with 5' terminally-labelled MboI fragments containing the 5'-end of either rRNA gene and total mtRNA. A: 5'-End 20S rRNA gene. S_1 nuclease-resistant hybrids were electrophoresed through a 2% alkaline agarose gel, blotted onto a nitrocellulose filter and visualized by autoradiography. Lanes a and b, S_1 nuclease-treated hybrids with 25 and 100 U/ml, respectively; c, input MboI fragment digested with MspI; d, input MboI fragment. B: 5'-End 15S rRNA gene. S_1 nuclease-resistant hybrids were electrophoresed through a 6% polyacrylamide-7 M urea gel and visualized by autoradiography. Lane a, input MboI fragment; b, input MboI fragment digested with HhaI; c and d, S_1 nuclease-treated hybrids with 50 and 100 U/ml, respectively. The lengths of S_1 nuclease-resistant hybrids are indicated. M denotes marker DNA, pBR322 x HinfI (see Methods).

end of this gene extends up to approximately 80 bp downstream the MspI site.

2. We determined the sequence of the 5'-end of the 20S rRNA. The in vitro capped 20S transcript was isolated from polyacrylamide gels and partially digested with ribonuclease T_1 to identify the positions of guanylyl residues extending from the 5'-end (Fig. 6). After a complete T_1 digestion the oligonucleotide of 20 nucleotides long was purified and sequenced enzymatically (Fig. 7).

The mapping of the 5'-end of 15S rRNA is based only on S_1 nuclease protection analysis (Fig. 5B), carried out as described for 20S rRNA using the 2300-bp MboI fragment, labelled at the 5'-end. Two S_1 nuclease-resistant fragments can be detected differing in quantity and in length by 40 nucleotides. We consider it likely that the fragment in low concentration (430 nucleotides long) results from hybridization with precursor RNA (15.2S), in analogy with the situation in S. cerevisiae. Thus, the 5' border of the 15S rRNA gene is located approximately 60 nucleotides downstream of the HhaI site (cf. Fig. 1).

In order to determine the DNA sequence around the positions at which both rRNAs start, parts of the original MboI inserts were sub-cloned into M13 (M-15A1 and M-20A1, see Fig. 1) and sequenced completely (see Methods). In clone M-20A1 (5'-end 20S

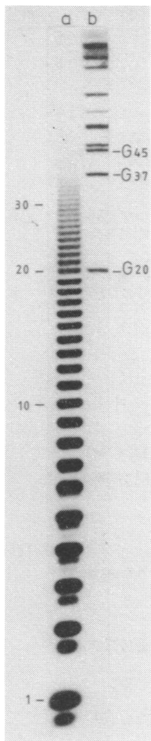

Fig. 6. Determination of the G-frame extending from the 5'-end of 20S rRNA. Lane a, Total capped mtRNA was partially digested with alkali. The resulting ladder was used to determine the size of the oligonucleotides in lane b. Lane b, 20S rRNA capped in vitro was isolated from a gel and partially digested with ribonuclease T1 which cleaves specifically after a G-residue. Electrophoresis was on a 20% polyacrylamide-8.3 M urea sequencing gel. The length of the first three oligonucleotides is indicated.

rRNA gene), the DNA sequence at the position predicted for S_1 nuclease analysis fits exactly with the sequence of the 5'-end of the 20S transcript (Fig. 7). Clone M-15A1 has been used to sequence the 5' region of the small rRNA gene. The S_1 nuclease experiment enables us to localize the 5'-end of both 15.2S and 15S rRNAs on the DNA sequence with an accuracy of about 2 nucleotides. We assume that the first nucleotide of 15.2S RNA is an A-residue, since initiation with other nucleotides is not found: complete digestion of total capped mtRNA, followed by

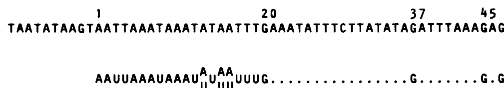

Fig. 7. Nucleotide sequence of the 5'-end of the large rRNA of *K. lactis* aligned with the DNA sequence (see also Fig. 6). Number 1 is the first nucleotide of the gene. The experimental evidence for the RNA sequence is not given.

Fig. 8. mtDNA sequences around the beginning of the rRNA genes in S. cerevisiae and K. lactis. Regions of homology are boxed. Arrows mark the 5'-ends of the rRNAs.

thin-layer chromatography (data not shown) shows that all radioactivity co-migrates with GpppA, indicating that all detectable mtRNA species are initiated with adenylic acid, as in S. cerevisiae [3].

An identical sequence of nine nucleotides precedes the rRNA genes

When we compare the DNA sequences around the beginning of both genes, we find the same nonanucleotide sequence at the same position with respect to the start of both rRNAs (Fig. 8). Moreover, the nucleotide sequence and its position are exactly the same as reported for S. cerevisiae [5]. This is not due to a general homology of nucleotide sequence in this region of the mtDNAs of the two strains. When the sequences around the begin-

Fig. 9. Comparison of DNA sequences from S. cerevisiae and K. lactis around the beginning of the genes for the large and small rRNAs. Positions of non-homology are indicated with x. Underlined are nucleotides forming part of the gene.

ing of the large and small rRNA genes are compared between *K. lactis* and *S. cerevisiae* (Fig. 9), numerous base substitutions and deletions are found surrounding this common sequence motif.

DISCUSSION

We have found that identical nucleotide sequences precede the genes where rRNA synthesis starts on mitochondrial DNA of *S. cerevisiae* and we proposed that these sequences may form part of a promoter for initiation of transcription [5]. The aim of this study was to analyse the initiation of rRNA synthesis on the mtDNA of the distantly-related yeast strain *K. lactis* to see if the principle of two separate promoters for the rRNA genes operates here also. Although the arrangement of the rRNA genes on *K. lactis* mtDNA is different [7,9] we find the same mode of transcription: the large rRNA is initiated *de novo* and subsequent processing at the 5'-end is unlikely since the position in the DNA sequence where the RNA starts is the same whether determined from S_1 nuclease protection experiments or from the terminal sequence of the 5'-end of the capped RNA. This is in line with the absence of processing at the 5'-end of large rRNA in *S. cerevisiae* [3]. Indirect evidence supports the idea that the small rRNA gene is transcribed as a precursor with an extension of 40 nucleotides at its 5'-end. S_1 nuclease protection experiments indicate where the beginning of the mature small rRNA can be found, but apart from the main protected fragment a 40-nucleotide longer protected fragment is found in much lower concentration. A minor in vitro capped RNA species hybridizes with DNA restriction fragments that contain the beginning of the small rRNA gene. Indeed, a 15.2S capped transcript is present in low amount (cf. Fig. 3), but we have not been able to perform sequence analysis on this RNA because of the high level of degradation products from the 20S rRNA. DNA sequence homology between the *K. lactis* and *S. cerevisiae* rRNA genes is good enough to indicate that in *K. lactis*, as in *S. cerevisiae* [4,11] we are dealing with a precursor RNA that is converted into mature 15S rRNA by processing at the 5'-end. We conclude that the mode of transcription of both rRNA genes is identical in these distantly-related yeasts.

We have determined the nucleotide sequence surrounding the positions at which the rRNAs start and comparison reveals that in K. lactis, as in S. cerevisiae, identical sequences immediately precede the start of rRNA synthesis. Moreover, part of the common sequence motif that we have found in S. cerevisiae in front of both rRNA genes is the same in K. lactis. Based on the combined sequence data from the four rRNA genes of these two yeasts, we believe that the most significant part is a nonanucleotide sequence nearest to the start of transcription although in each yeast the homology extends further upstream or downstream. We therefore consider this nonanucleotide sequence an important part of the promoter responsible for rRNA synthesis.

Apart from this striking homology our data indicate also some differences between the rRNA genes of the two strains. From the completely sequenced insert fragment of clones M-20A1 and M-15A1 (not shown) we can draw several conclusions. The 5'-ends of the large rRNAs examined over a length of 70 nucleotides show a high degree of homology (circa 95%) except for the extreme 5'-end (cf. Fig. 9). The mature RNAs differ in length by approximately 400 nucleotides as determined from agarose gel electrophoresis (Fig. 3). The mature small rRNAs do not differ in size and show about 90% identity over the first 390 nucleotides (compared with ref. 18). The 5' leader is significantly shorter in K. lactis primarily due to deletions of 27 and 7 nucleotides (cf. Fig. 9) but processing seems to be the same in both strains. A further difference is the presence of a GC-rich cluster in the mature part of the gene (220 bp downstream of the processing site) which is absent from the S. cerevisiae strain used. S_1 nuclease protection experiments have shown that this GC cluster is transcribed and conserved in the mature small rRNA. The same type of variation in small rRNA genes has been observed between several S. cerevisiae strains. The GC cluster in K. lactis, however, is present at a position other than the one found in the S. cerevisiae strains [19].

We have inspected published DNA sequences comprising about 60% of the total mitochondrial genome of S. cerevisiae for the presence of the identified nonanucleotide sequence and found this motif in some other positions. One is present in front of

the gene coding for ATPase subunit 9 at a position where the 5'-end of 12S messenger RNA for this protein ends as determined from S_1 nuclease analysis (Janssens, P.M. and Hensgens, L.A.M., personal communication) and we have indications that initiation of transcription takes place at this position.

In addition, it is of interest that the nonanucleotide sequence occurs in petite mutants that share the property of hypersuppressiveness [20-22]. Such petite mutants when crossed with a wild-type strain, lead to zygotic clones composed almost entirely of ρ^- cells with the original hypersuppressive mtDNA, presumably because wild-type mtDNA is excluded from the descendants of the zygote. The repeat units of hypersuppressive petites share a 300-bp region with extensive sequence homology, which is considered to be an origin of DNA replication. The explanation put forward for hypersuppressiveness is that such petite mutants preferentially replicate their mtDNA after zygote formation at the cost of wild-type mtDNA, because they contain multiple copies of a highly efficient replication origin [20-22]. Several regions of the wild-type mitochondrial genomes contain such replication origins and the structure of these is shown in Fig. 10. Not only is the nonanucleotide sequence present in the 300-bp region of hypersuppressive petite mutants in a characteristic position with respect to one of the three conserved GC clusters, but it is also functional in promotion of RNA synthesis:

Fig. 10. Schematic presentation of the 300-bp region common to hypersuppressive petites. In the upper part of the figure, GC clusters 1-3 are boxed and a, b, c and d are AT-rich stretches. a is either 3 or 17 bp long, while b, c and d are approx. 200, 25 and 20 bp, respectively. The nonanucleotide sequence is underlined. The arrow indicates the initiation of RNA synthesis. The lower part shows the sequence of four hypersuppressive petite mutants, extending from the nonanucleotide sequence to GC cluster 1 (underlined). Sequence data are taken from ref. 20 (a) and ref. 22 (b-d). The sequence from (c) is part of the replication origin identified between oli2 and cytochrome b.

hypersuppressive petite mutants still contain mtRNA [23] and more recently we have shown (unpublished) that the 5'-terminal sequence of one cappable RNA, isolated from petite mutants retaining a segment of the genome between oli2 and cytochrome b, can be perfectly aligned with the sequence of the replication origin identified in this region. The alignment demonstrates a start within the nonanucleotide box. We conclude that at least one nonanucleotide sequence in such a replication origin is used for initiation of transcription and it is possible that it functions in RNA priming of mtDNA replication. In this context it is worthy of note that homology exists between elements of the putative yeast origins of replication (GC clusters) and the origin of H-strand synthesis in human mitochondria [22]. The direction of RNA synthesis, initiated from the nonanucleotide box, is the same as the known direction of the H-strand replication [24] and this is consistent with the function we propose. If so, we have to assume that many priming events in petite mutants do not lead to DNA replication, but to continuation of RNA synthesis possibly due to a limited capacity of enzyme(s) involved in synthesis of DNA.

De Zamaroczy et al. [22] have proposed that at least seven replication origins exist on wild-type mtDNA. Only a sub-set of the petite mutants retaining these origins, however, are hypersuppressive and contain the nonanucleotide sequence. The remainder shows lower and variable degrees of suppressiveness and were selected primarily by restriction enzyme analysis. Sequence analysis shows that they lack the nonanucleotide box.

The possibility that the same promoter and, therefore, the same mtRNA polymerase is used both for DNA replication and for RNA synthesis, raises the interesting possibility that by controlling the synthesis of mtRNA polymerase the nucleus can regulate mitochondrial proliferation at the level of DNA replication and protein synthetic capacity.

ACKNOWLEDGEMENTS

We thank Professors P.Borst and L.A.Grivell for helpful discussions. KAO was the recipient of a Short-Term Fellowship from EMBO and is grateful to Professor M.Rabinowitz (Chicago, Ill., USA). for his hospitality. We are indebted to Mr. R.F.Evers for expert technical assistance and to Prof. J.H.Van Boom (State University Leiden) for a gift of a synthetic primer DNA for M13 sequence analysis. TC was supported by a United States Public Health

Service grant (HL-04442) to M.Rabinowitz. This work was supported in part by a grant to P.Borst/L.A.Grivell from The Netherlands Foundation for Chemical Research (SON) with financial aid from The Netherlands Organization for the Advancement of Pure Research (ZWO).

Abbreviations: bp, base pair(s); kb, kilo-base pair(s); rRNA, ribosomal RNA.

REFERENCES

1 Tabak, H.F., Grivell, L.A. and Borst, P. (1982) in: CRC Critical Reviews in Biochemistry (Fasman, G.D., Ed.), CRC Press, Boca Raton, 1982, in press.
2 Slonimski, P., Borst, P. and Attardi, G. (Eds) (1982) Mitochondrial Genes, Cold Spring Harbor Laboratory, Cold Spring Harbor,N.Y.
3 Levens, D., Ticho, B., Ackerman, E. and Rabinowitz, M. (1981) J.Biol. Chem. 256, 5226-5232.
4 Christianson, T., Edwards, J., Levens, D., Locker, J. and Rabinowitz, M. (1982) J.Biol.Chem. 257, 6494-6500.
5 Osinga, K.A. and Tabak, H.F. (1982) Nucl.Acids Res. 10, 3617-3626.
6 Sanders, J.P.M., Weijers, P.J., Groot, G.S.P. and Borst, P. (1974) Biochim.Biophys.Acta 374, 136-144.
7 Groot, G.S.P. and Van Harten-Loosbroek, N. (1980) Curr.Gen. 1, 133-135.
8 Groot, G.S.P. and Van Harten-Loosbroek, N. (1980) in: Organization and Expression of the Mitochondrial Genome (Kroon, A.M. and Saccone, C., Eds), North-Holland, Amsterdam, pp. 61-64.
9 Wésolowski, M., Algeri, A. and Fukuhara, H. (1981) Curr.Gen. 3, 157-162.
10 Groot, G.S.P., Flavell, R.A. and Sanders, J.P.M. (1975) Biochim.Biophys. Acta 378, 186-194.
11 Osinga, K.A., Evers, R.F., Van der Laan, J.C. and Tabak, H.F. (1981) Nucl.Acids Res. 9, 1351-1364.
12 Bolivar, F., Rodriguez, R.L., Betlach, M.C. and Boyer, H.W. (1977) Gene 2, 75-93.
13 Grunstein, M. and Hogness, D.S. (1975) Proc.Nat'l.Acad.Sci.U.S. 72,3961-3965.
14 Messing, J., Crea, R. and Seeburg, P.H. (1981) Nucl.Acids Res. 9,309-321.
15 Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc.Natl.Acad.Sci. U.S. 74, 5463-5467.
16 Sanger, F., Coulson, A.R., Barrell, B.G., Smith, A.J.H. and Roe, B.A. (1980) J.Mol.Biol. 143, 161-178.
17 Silberklang, M., Gillum, A.M. and RajBhandary, U.L. (1979) in: Methods in Enzymology (Moldave, K. and Grossman, L., Eds), Vol. 59, Academic Press, New York, pp. 58-109.
18 Sor, F. and Fukuhara, H. (1980) Compt.Rend. Sér. D 291, 933-936.
19 Sor, F. and Fukuhara, H. (1982) Nucl.Acids Res. 10, 1625-1633.
20 Blanc, H. and Dujon, B. (1980) Proc.Natl.Acad.Sci.U.S. 77, 3942-3946.
21 Blanc, H. and Dujon, B. (1982) in: Mitochondrial Genes (Slonimski, P., Borst, P. and Attardi, G., Eds), Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., pp. 279-294.
22 De Zamaroczy, M., Marotta, R., Faugeron-Fonty, G., Goursot, R., Mangin, M., Baldacci, G. and Bernardi, G. (1981) Nature 292, 75-78.
23 Bernardi, G. (1982) in: Mitochondrial Genes (Slonimski, P., Borst, P. and Attardi, G., Eds), Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., pp. 269-278.
24 Crews, S., Ojala, D., Posakony, J., Nishiguchi, J. and Attardi, G. (1979) Nature 277, 192-198.
25 Sanders, J.P.M., Heyting, C., Verbeet, M.Ph., Meijlink, F.C.P.W. and Borst, P. (1977) Mol.Gen.Genet. 157, 239-261.