Nucleic Acids Research, Vol. 20, No. 17 ***4445–4450***

Gene dosage as a possible major determinant for equal expression levels of genes encoding RNA polymerase subunits in the hypotrichous ciliate *Euplotes octocarinatus*

Jörg Kaufmann and Albrecht Klein*
Molecular Genetics, Department of Biology, Philipps University, PO Box 1929, W-3550 Marburg, Germany

Received June 17, 1992; Revised and Accepted August 3, 1992 EMBL accession nos X66450–X66453 (incl.)

ABSTRACT

Ciliated protozoa harbor two different types of nuclei in each cell. The diploid micronucleus is the transcriptionally inactive generative nucleus, while the macronuclous contains a highly amplified transcriptionally active genome of lower complexity. The macronuclear genes encoding the two largest subunits of both RNA polymerases I and II of *Euplotes octocarinatus* were identified by a novel method of two step PCR walking, employing primer pairs derived from telomeric sequences of the organism and known conserved RNA polymerase polypeptide sequences, respectively. The relative gene dosage was determined. The genes are present in equal copy numbers for the respective matching subunits. Northern hybridizations showed comparable amounts of transcripts, as well, within the matching pairs. Mapping of the 5′-termini of the transcripts of the gene sized chromosomes showed that the upstream nontranscribed regions are very short and contain characterisitic sequence motifs which could be the determinants of equal promoter strengths for subunits of a common RNA polymerase.

INTRODUCTION

Ciliated protozoa are characterized by nuclear dimorphism. The two different types of nuclei present in each cell serve different functions. The generative micronucleus comprises the complete genetic information in transcriptionally inactive form, while the vegetative macronucleus contains functional chromosomes in high copy numbers (1). In the case of hypotrichous ciliates like *Euplotes* these chromosomes comprise one gene each (2), with the probable exception of rRNA genes (3) which are linked on one chromosome. They are bounded by telomeres.

The sequence complexity of the macronuclear genome is greatly reduced compared with that of the diploid micronucleus (1). This loss of sequence information as well as the amplification of the residual genes occurs during macronucleus development from a micronucleus after a sexual process called conjugation. The copy numbers of the different macronuclear chromosomes are different, ranging from 10^3 to 10^5 per macronucleus (4, 5). They are species specific and are kept constant during vegetative cell divisions (6, 7). Therefore, the dosage of different genes is fixed. This could serve as a basic mechanism of determining the amounts of gene products, especially for house keeping genes, and make involved transcriptional control unnecessary in these cases. Such a mechanism would be of special advantage in the cases of subunits of multicomponent proteins.

In order to test this notion we have analyzed the relative gene copy numbers and amounts of transcripts of two pairs of genes encoding RNA polymerase subunits, whose stoichiometric amounts in the enzyme are known (8, 9). We report that, indeed, the copy numbers of the macronuclear chromosomes carrying the genes encoding the two largest subunits each of RNA polymerases I and II, RPA1 and RPA2, or RPB1 and RPB2, respectively, are present in equal copy numbers and that the genes belonging to each pair are transcribed at comparable levels.

The very short nontranscribed regions as defined by mapping of the 5′-ends of the transcripts show common features close to and/or upstream of the transcription start sites for each of the two pairs of the matching genes belonging to the different polymerases.

MATERIALS AND METHODS

DNA cloning, sequencing, nucleic acid hybridization, and polymerase chain reactions (PCR)

Restriction analysis, DNA cloning after GC-tailing or insertion of restriction fragments into pT7T3 plasmid vectors (Gibco/BRL), DNA hybridization, PCR, and sequencing of cloned or amplified DNA according to the dideoxy chain termination method was performed as described (10, 11). Direct sequencing (12) of PCR products was carried out after their elution from agarose gels.

Ciliate cultures and macronucleus preparation and lysis

Euplotes octocarinatus strain 2 (68)-VIII used in this study was obtained from K.Heckmann, Münster and grown on *Chlorogonium elongatum* (Algal Strain Collection, Göttingen) as described (13). Macronuclei were prepared as previously described (14) after concentrating the cells by filtration through nylon gauze (60 μm pore size), and subsequent cell lysis and size

* To whom correspondence should be addressed

Table 1. Primers used in PCR-amplification

	Primer peptide	derived DNA sequence
1	YNADFDGDEMN	GTTCATTTCGGATCC(AG)TCGAA(AG)TCAGCGTT(AG)TA[1]
2	MGKRVDFSAR	ATGGG(AT)AA(GA)AG(AT)GTGGAATTCTCATCAGC(TA)AGA
3	PIMLRSXXC	(GA)CAGGATCCAGATC(GT)TA(AG)CAT(AT)AT(AT)GG[1]
4	NPHXXPSRMT	GTCATTCT(AT)(CG)(TA)(AT)GGATCCGC(AG)TG(TA)GGATT[1]
5	PDXNQSPRN(MT)YQ	CC(AT)GAATTCAA(CT)CAA(TA)G(AT)CC(AT)AG(AG)AATA(TC)(TG)TATCAA
6	GGYF(VI)(VI)NGSE(KR)	GG(AT)GG(AT)TATTTTAT(TA)AT(TA)AATGG(AT)(AT)(CG)AGAA
Telo[2]	CCAAAACCCCGGATCCCCAAAACCCC	CCCAAAACCCGAATTCCCCAAAACCCCAAA

[1]Antisense sequence. [2]The Telo oligonucleotides were derived from the 5'-recessed end sequence of the telomeres. They differ in the inserted BamHI or EcoRI restriction sites and were used according to the restriction sites used in the cloning vector. Restriction sites incorporated into the primers sequences are shown in bold letters.

fractionation of the released nuclei by filtration through nylon gauze (10 μm pore size) and sedimentation at 100 g for 15 min at 4°C. The nuclei were lysed at a concentration of $1-3\times10^6$/ml in 10 mM Tris/HCl pH 9.5, 0.5 M EDTA, 1% SDS at 65°C for 15 min followed by the addition of a final concentration of 2 mg/ml of proteinase K and further incubation for 16 h at 50°C.

RNA preparation

RNA was obtained from whole cells according to the one step extraction method (15) employing acidic guanidinium thiocyanate and phenol extraction.

PCR walking

Polymerase chain reactions were carried out with Taq DNA polymerase (Perkin-Elmer/Cetus) which was used according to the supplier's instructions. Macronuclear DNA was size fractionated in the range of the expected gene length prior to its use as template at 1 μg/ml in 100 μl reactions. Primers derived from conserved eukaryotic RNA polymerase oligopolypeptide sequences (16–23) were synthesized on an Applied Biosystems model 391 synthesizer. They are given in Table 1. They were first used, partially in conjunction with primers derived from the telomeric sequences. The optimal temperatures for the hybridization steps during the PCR were optimized for each primer pair. After the primary amplification using such primer pairs, secondary primers were derived from partially sequenced amplification products. The primer combinations applied for the individual reactions are specified in Figure 1 of the Results section.

Quantitative Southern blots

^{32}P-labeled (24, 25) homologous probes were employed under stringent conditions for quantitative Southern blots in the determination of gene copy numbers of the different genes encoding RNA polymerase subunits. The specific activities of the probes were measured by parallel hybridizations against known amounts of cloned homologous DNAs. In addition, a specific mixed oligonucleotide derived from a consensus sequence in the 5'-regions of the RPB1 and RPB2 genes was employed for another independent copy number determination of these genes.

Northern blots and estimation of the relative steady state amounts of RNA polymerase transcripts

The estimation of the relative amounts of RNA polymerase transcripts was performed on total RNA transferred and bound to nylon filters (Biodyne B, Pall) after its electrophoretic separation on 1.2% formaldehyde agarose gels. ^{32}P-labeled gene specific probes of comparable specific activities were employed.

Primer extensions

^{32}P-labeled primers were hybridized to 10–50 μg total RNA and reverse transcription was performed at 42°C with AMV reverse transcriptase (Boehringer) used according to the supplier's instructions. The products were analyzed on a denaturing 6% polyacrylamide gel employing sequence markers as size standards.

RESULTS

Identification of macronuclear RNA polymerase genes in *Euplotes octocarinatus*

The previous comparison of genes encoding eukaryotic RNA polymerase subunits has yielded conserved amino acid sequences in both of the largest subunits of all three types of the enzyme, RNA polymerases I, II and III. In order to assess if such sequences would be at all useful for the search for RNA polymerase structural genes in ciliates Southern blots of macronuclear DNA of *E.octocarinatus* and *Stylonychia lemnae*, both fresh water inhabiting hypotrichous ciliates, were probed with an oligonucleotide derived from the most common polypeptide of the largest subunit of the polymerases (YNADFDGDEMN, 16–18). In both cases one strong signal at 5 kb was detected which was the presumed size for macronuclear chromosomes comprising only the respective genes and the expression signals. This was as expected as no cases are known in hypotrichs, in which one macronuclear chromosome carries more than one protein encoding gene. This result encouraged us to use the strategy outlined in Figure 1 to look for both, the genes encoding the largest and second largest subunits of different RNA polymerases in *E.octocarinatus*. Overlapping gene fragments were produced by PCR walking employing heterologous primers (derived from conserved amino acid sequences of the homologous gene products from other organisms) together with primers derived from the telomere sequence or in conjunction with homologous primers derived from partial sequences of previously isolated fragments.

In all cases in which telomere primers were used, size fractionation of the macronuclear DNA was performed after identification of the macronuclear chromosomes in Southern hybridization experiments in order to avoid amplification of complete shorter chromosomes end to end.

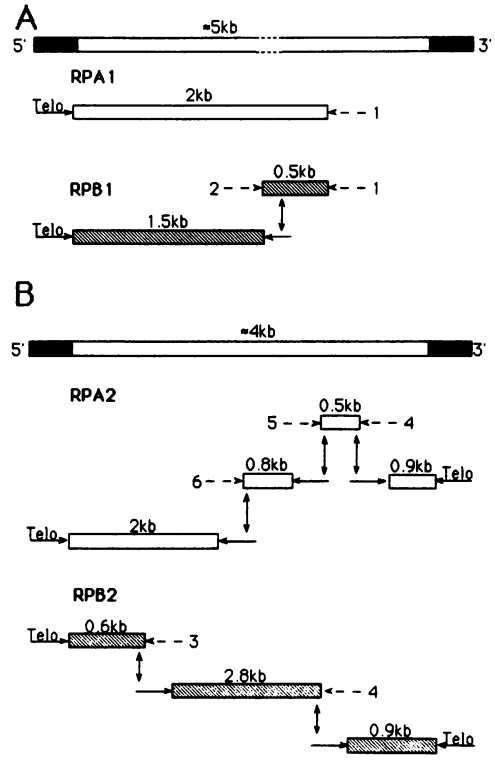

Figure 1. PCR walking strategy for the isolation of macronuclear RNA polymerase gene sequences. (A) RPA1 and RPB1 sequences, encoding N-terminal parts of the largest subunits of RNA polymerases I and II, respectively. The size of the macronuclear chromosomes, bounded by telomeric sequences (filled ends) is indicated at the top of the figure. The fragments of the RPA1 (open bar) and RPB1 (hatched bars) genes obtained are shown in the lower part. Solid arrows indicate homologous primers, while heterologous primers are indicated by broken arrows. They are numbered in accordance with the Materials and Methods section. Vertical double headed arrows indicate the regions from which homologous primers were derived for secondary walking steps. Telomeres and primer lengths are not drawn to scale. (B) Genes RPA2 and RPB2, encoding the second largest subunits of the same two RNA polymerases. The signatures are analogous to those in (A).

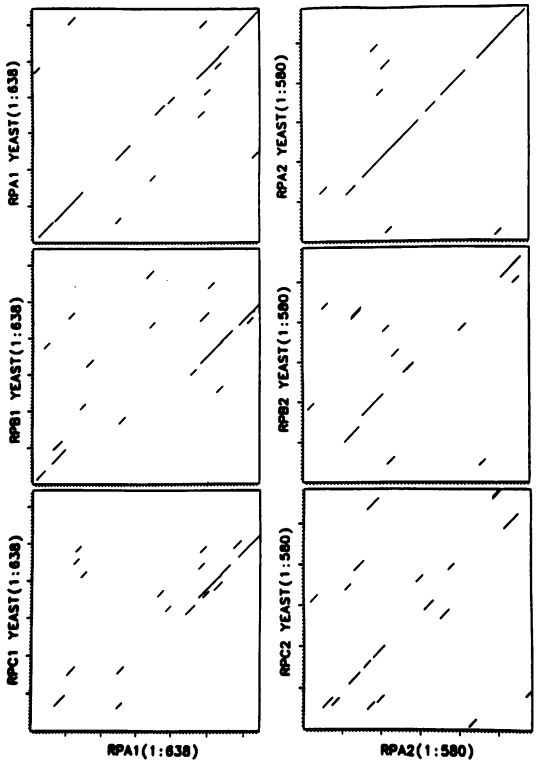

Figure 2. Identification of genes RPA1 and RPA2, encoding the two largest subunits of RNA polymerase I. Derived N-terminal polypeptide sequences of the lengths given at the axes of the dot matrices were compared with known sequences of the respective genes of RNA polymerases I, II or III (called RPA1, RPB1, RPC1, RPA2, RPB2, and RPC2, for simplification) of *Saccharomyces cerevisiae*. Their original names are RPA1 = RPA190 (16), RPB1 = RP021, RPC1 = RP031 (39), RPA2 = RPA135 (22), RPB2 (20), and RPC2 = RET1 (23), respectively.

Identification of the different polymerase gene types

In order to specify, to which RNA polymerase type the identified genes belonged, N-terminal sequences were derived from the sequences of the 5'-terminal gene fragments and compared to known sequences of the respective genes from *Saccharomyces cerevisiae*. Figures 2 and 3 document that the genes of the two largest subunits of each of the RNA polymerases I and II, RPA1 and RPA2, as well as RPB1 and RPB2 had been identified. The figures also show that the relationship between polymerases II and III appears closer than that among polymerase I and the other two enzymes, which is in line with previous observations (18, 26)

Determination of the relative copy numbers of the genes encoding pairs of RNA polymerase subunits

The control of differential gene amplification which apparently operates during macronuclear development of hypotrichous ciliates would allow the adjustment of the gene dosage to the required stoichiometry of the needed products in multisubunit protein complexes such as the RNA polymerases where the two largest subunits are present at a 1:1 ratio both in bacteria (8) and eukaryotes (9). In order to test this notion quantitative Southern hybridizations were carried out on macronuclear DNA of *E.octocarinatus* with mixtures of gene probes derived from the two genes encoding the two largest subunits of RNA polymerases I or II, respectively. The results are shown in Figure 4. It is obvious in both cases that the two genes do indeed occur in equal copy numbers, i.e. at the same amplification levels, in the macronucleus. Scanning the signals of the left RPA lane yielded a ratio of 0.46 (lower to upper signal), which matches the ratio of the probe lengths of 0.9 to 2.0 kb.

Assessment of the relative amounts of transcripts of the identified RNA polymerase I and II subunit genes

Northern blots were obtained from total RNA of *E. octocarinatus* and hybridized against an excess of cloned and radioactively labeled gene sequences of the homologous genes. We did not isolate a polyA−RNA fraction in order to avoid an artifactual distortion of the ratio of the transcripts. Due to the relatively small fraction of the respective mRNAs in the total RNA the signals were weak and allow only an estimate of the ratios of the transcripts. The result shown in Figure 5 represents one of several experiments using RPA and two experiments using RPB specific probes. For RPA the amount of the transcripts appears to be in the same order of magnitude. An equal amount of the transcripts cannot be taken from this experiment. Therefore it remains unclear whether the ratio of the two transcripts is directly proportional to the ratio of the gene copy numbers in this case.

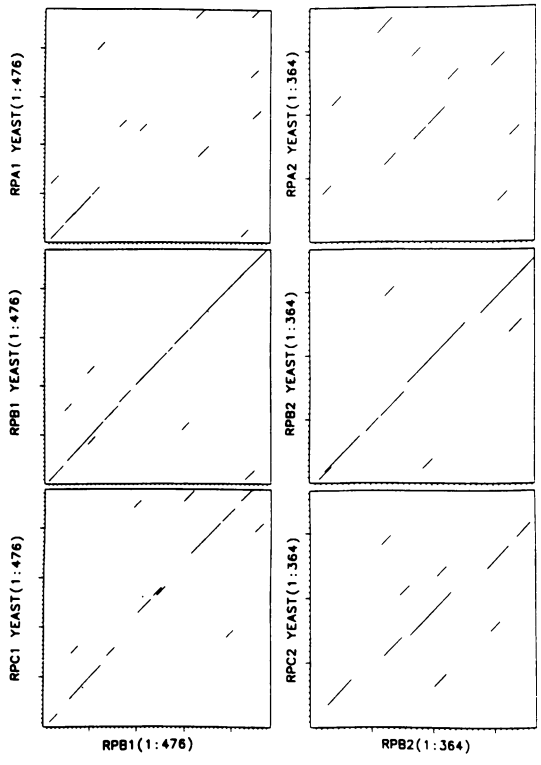

Figure 3. Identification of genes RPB1 and RPB2, encoding the two largest subunits of RNA polymerase II. Derived N-terminal polypeptide sequences of the lengths given at the axes of the dot matrices were compared with known sequences of the respective genes of all three RNA polymerases of *Saccharomyces cerevisiae*. The nomenclature was chosen as in Figure 2.

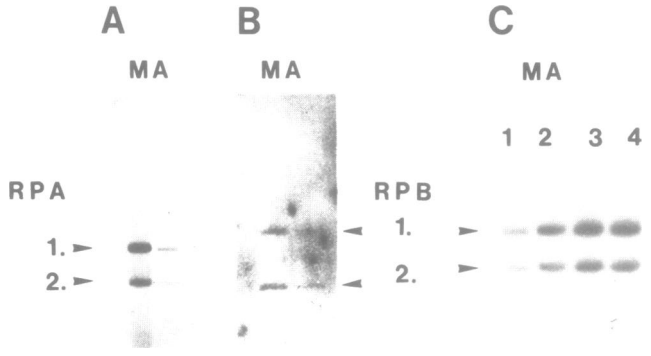

Figure 4. Autoradiograms of Southern blots of 10 μg (left lanes) or 1 μg (right lanes) of macronuclear DNA probed with homologous 5′-terminal gene probes of genes RPA1 and RPA2 (A) or RPB1 and RPB2 (B). Note that the lengths of the equally labeled homologous probes were different, which explains the apparent different signal strengths for RPA1 and RPA2. RPA1, 2 kb; RPA2, 0.9 kb, RPB1, 0.5 kb; RPB2, 0.6 kb. (C) Autoradiogram of a Southern blot of 1, 5, 10, or 20 μg macronulcear DNA (left to right) probed with a common mixed oligonucleotide probe derived from the 5′-terminal regions of genes RPB1 and RPB2 which comprises a fully conserved undecanucleotide (compare Figure 7).

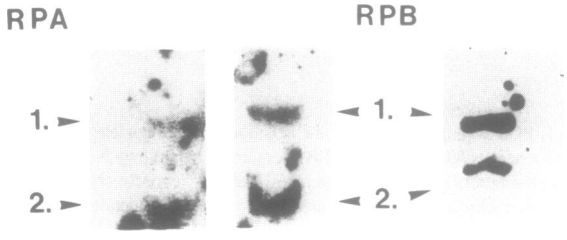

Figure 5. Autoradiogram of a Northern hybridization of total RNA from vegetative *E.octocarinatus* cells. Left side track: RPA1 and RPA2 transcripts; right side tracks: RPB1 and RPB2 transcripts from two independent experiments. Homologous gene sequences of comparable total lengths cloned in pT7T3α-18 were used as probes under stringent conditions.

For RPB the results of the two experiments shown suggest that this might be true, although the comparison of the two experiments also shows the degree of uncertainty of this conclusion.

Analysis of the subtelomeric regions preceding the 5′-ends of the identified RNA polymerase subunit genes

Macronuclear chromosomes of *Euplotes* have characteristic telomeric sequences and consensus sequences for putative fragmentation sites used to process the micronuclear precursors into macronuclear chromosomes (27). They occur at a distance of 17 bp from the proximal telomeric repeat. It is therefore easy to confirm that a clone obtained by PCR amplification at the 5′-end of a macronuclear gene does contain the terminal region and has not resulted from unspecific priming closer to the beginning of the gene. Figures 6 and 7 show the sequences of the 5′-subtelomeric regions preceding the four analyzed genes. Using the indicated primers, the 5′-ends of the transcripts were mapped to the indicated nucleotides, which are in all but one case located on an A residue in the proximity of one or two nucleotides to a C residue within a consensus sequence for mRNA capping (28, 29). The indicated alternative 5′-ends could be artifacts, in which case the most telomere proximal terminus would have to be assumed to be the correct one. However, examples of multiple staggered initiation points have previously been described (30, 31). It is striking that all nontranscribed regions are very short. In both the RPB1 and RPB2 genes TATA box sequences can be seen in the expected distances upsteam of the mapped 5′-end of the transcript which is most proximal to the telomere. In addition, in these cases a conserved sequence (13 out of 14 identical nucleotides) is detected close to the putative transcription start sites (Figure 7). As also noticed in the case of the homologous yeast genes (16, 22) TATA box sequences are missing in front of the transcription start sites of the two RPA genes. However, the most telomere proximal 5′-termini are immediately preceded by stretches of 19 or 17 AT pairs in the RPA1 and RPA2 genes, respectively, which have been recognized as upstream promoter elements of constitutively transcribed yeast genes (32, 33). CCAAT containing motifs have been described in front of genes of ciliated protozoa, mostly in *Tetrahymena* (34). They are not found in front of the *Euplotes* genes described here, which is not surprising since they are usually located at a distance in front of the transcription start, which exceeds the available DNA stretches in front of the *E.octocarinatus* RNA polymerase genes.

DISCUSSION

In the prokaryotic organisms studied so far the homologs of the genes encoding the two largest subunits in the eukaryotic RNA polymerases are organized in polycistronic transcription units, which facilitates the stoichiometric production of the proteins (35).

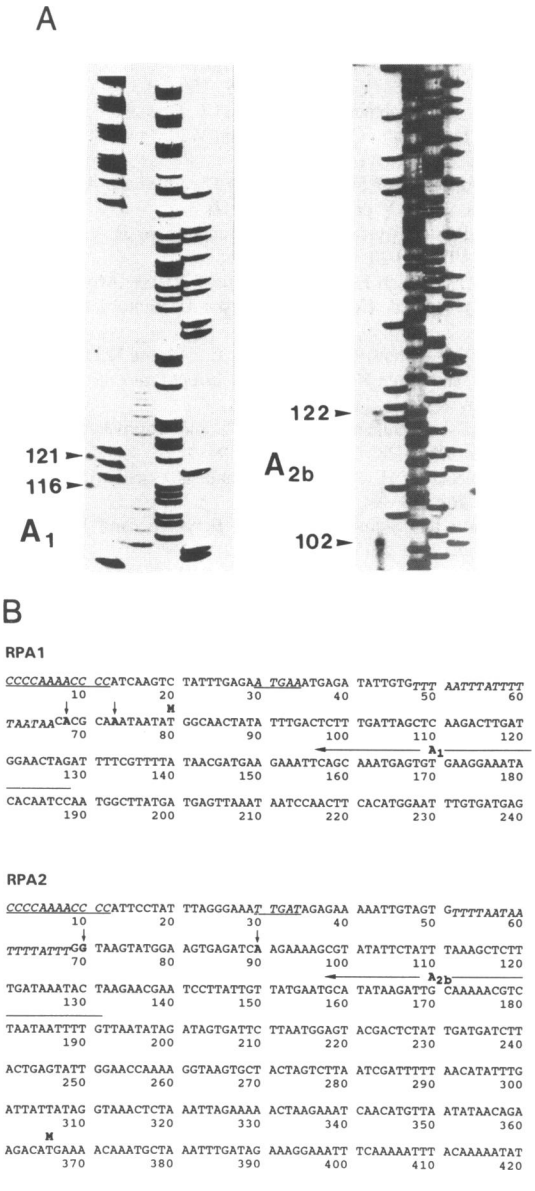

Figure 6. (A) Mapping of the 5'-termini of the transcripts encoding the largest and second largest subunits of RNA polymerase I by primer extensions. The primers used (A_1 and A_{2b}) are shown next to the signals and as horizontal arrows in (B). The primer extension signals are shown in the left lanes. The adjacent size markers do not represent the RNA polymerase gene sequences. The necessity of application of large amounts of RNA caused the narrowing of the tracks showing the extension signals as compared to the size markers on the same gel. The numbered arrows indicate the lengths of the signals. (B) Sequences of the 5'-ends of the RPA1 (top) and RPA2 genes (bottom). Telomeric sequences at the 5'-ends and consensus fragmentation sequences 17 bp downstream are indicated in italics and underlined. Vertical arrows point to the terminal nucleotides of the transcripts. The potential N-terminal methionine residues of the polypeptides are shown in bold face above the nucleotide sequences. Due to the lack of available N-terminal peptide sequences they had to be assumed on the basis of sequence comparisons as shown in Figures 2 and 3. Conserved extended AT-tracts directly upstream of the transcription starts are shown in subscript italics.

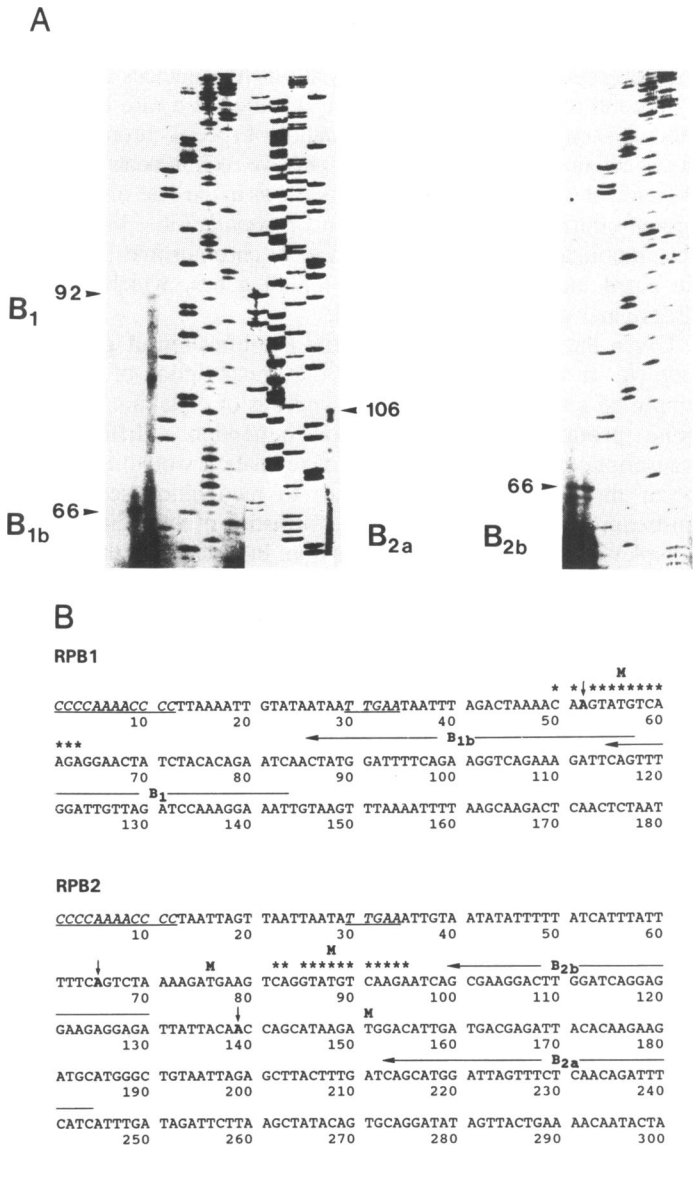

Figure 7. (A) Mapping of the 5'-termini of the transcripts encoding the largest and second largest subunits of RNA polymerase II. The primers used for mapping of the 5'-ends of the transcripts (B_{1b} or B_{2a} and B_{2b}) are shown next to the signals and as horizontal arrows in (B). The primer extension signals are shown in the left lanes. The adjacent size markers do not represent the RNA polymerase gene sequences. The necessity of application of large amounts of RNA caused the narrowing of the tracks showing the extension signals as compared to the size markers on the same gel. The numbered arrows indicate the lengths of the signals. (B) Sequences of the 5'-ends of the RPB1 (top) and RPB2 genes (bottom). Telomeric sequences at the 5'-ends and consensus fragmentation sequences 17 bp downstream are indicated in italics and underlined. Vertical arrows point to the terminal nucleotides of the transcripts. The potential N-terminal methionine residues of the polypeptides are shown in bold face above the nucleotide sequences. Due to the lack of available N-terminal peptide sequences they had to be assumed on the basis of sequence comparisons as shown in Figures 2 and 3. Conserved extended AT-tracts directly upstream of the transcription starts are shown in subscript italics.

In contrast, the respective eukaryotic genes can be completely unlinked and must be separately expressed. In haploid or diploid organisms this requires the regulation of the coordinate expression of the matching gene pairs on the transcriptional level.

The results presented here indicate that in hypotrichous ciliates such as *E.octocarinatus* a basic precondition for equal expression of the two largest RNA polymerase subunit genes can be achieved by equal amplification of the gene sequences of each matching pair of both RNA polymerases I and II. Hypotrichous ciliates have a unique general possibility of the predetermination of ratios

of transcripts on the basis of template amounts, since their macronuclear chromosomes are all differentially amplified during the generation of this transcriptionally active nucleus type.

In contrast, differential gene amplification is a rare event in other eukaryotes. Temporal amplification of special chromosomal genes is found in cases of particular massive requirements of gene products at a certain developmental stage as in the case of chorion proteins during egg development in *Drosophila* (36–38), while the amplification of the genome in giant chromosomes leads to an equal increase of the dosage of all genes, which entails differential transcriptional control.

Given the potential of differential amplification of genes in *Euplotes* it appears very unlikely that transcription of equally amplified genes required for the synthesis of equal amounts of gene products is under factor dependent, differential transcriptional control. This type of regulation would normally occur through binding of proteins to recognition sequences upstream of those necessary for the formation of the preinitiation complex required for basic transcription. In the cases of the genes described here the nontranscribed regions are indeed very short and canonic sequences implicated in factor binding, such as the CCAAT motif are not found. Enhancers within the genes cannot be ruled out, whereas the 3'-noncoding regions of the genes are also very short (data not shown).

We would therefore assume that the promoter strengths determined only by the DNA sequences close to the transcription start sites govern the transcription rates of the four RNA polymerase genes studied here. This implies that the upstream regions of equally expressed genes should have common features determining equal promoter strength, which does not require sequence identity. This is supported by our finding that the subtelomeric regions preceding the transcribed parts of the macronuclear chromosomes carrying the identified RNA polymerase genes exhibit common features within the two pairs of equally expressed genes. Our estimates of the ratios of the amounts of transcripts from the two gene pairs on the basis of Northern blots do not allow a firm conclusion as to whether steady state concentrations of the transcripts have the same ratios as the gene copy numbers. They appear to be in the same order of magnitude. However, even if they could be shown to be equal, it would not be excluded that the steady state levels are the result of the compensation of different synthesis rates by differential degradation of the transcripts. Even though we consider it unlikely, this would have to be ruled out by future studies on the transcription rates and/or stabilities of the RNAs.

ACKNOWLEDGEMENTS

We want to acknowledge fruitful discussions with Volker Florian and Erika Kothe, whom we also thank for carefully reading the text. Thanks are due to Hannelore Steinebach for her help with the preparation of the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie.

REFERENCES

1. Klobutcher,L.A. and Prescott,D.M. (1986) In Gall,J.G. (ed.), *The Molecular Biology of Ciliated Protozoa*. Academic Press, Inc., Orlando, Florida, pp. 111–114.
2. Swanton,M.T., Heumann,J.M. and Prescott,D.M. (1980) *Chromosoma* **77**, 217–227.
3. Swanton,M.T., Greslin,A.F. and Prescott,D.M. (1980) *Chromosoma* **77**, 203–215.
4. Wünning,I.U. and Lipps,H.J. (1983) *EMBO J.* **2**, 1753–1757.
5. Steinbrück,G. (1983) *Chromosoma* **88**, 156–163.
6. Baird,S.E. and Klobutcher,L.A. (1991) *J. Protozool.* **38**, 136–140.
7. Larson,D.D., Umthun,A.R. and Shaiu,W.-L. (1991) *J. Protozool.* **38**, 258–263.
8. Chamberlin,M. (1982) In Boyer,P. (ed.) *The Enzymes*. Academic Press Inc., New York. Vol XV part B. pp. 61–86.
9. Kolodziej,P.A., Woychik,N., Liao,S. and Young,R.A. (1990) *Mol. Cell. Biol.* **10**, 1915–1920.
10. Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
11. Ausubel,F.M., Brent,R., Kingston,R.E., Moore,D.D., Seidmann,J.G., Smith,J.A. and Struhl,K. (eds.) (1987) *Current Protocols Molecular Biology*. John Wiley & Sons, Inc., New York.
12. Casanova,J.-L., Pannetier,C., Jaulin,C. and Kourilsky,P. (1990) *Nucleic Acids Res.* **18**, 4028.
13. Schulze-Dieckhoff,H., Freiburg,M. and Heckmann,K. (1987) *Eur. J. Biochem.* **168**, 89–94.
14. Ammermann,D., Steinbrück,G., von Berger,L. and Hennig,W. (1974) *Chromosoma*, **45**, 401–429.
15. Chomczynski,P. and Sacchi,N. (1987) *Anal. Biochem.* **162**, 156–159.
16. Mémet,S., Gouy,M., Marck,C., Sentenac,A. and Buhler,J.-M. (1988) *J. Biol. Chem.* **263**, 2830–2839.
17. Cornelissen,A.W.C.A., Evers,R. and Köck,J. (1988) *Oxford Surveys Euk. Gen.* **5**, 91–131.
18. Pühler,G., Leffers,H., Gropp,F., Palm,P., Klenk,H.-P., Lottspeich,F., Garrett,R.A. and Zillig,W. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 4569–4573.
19. Falkenburg,D., Dworniczak,B., Faust,D.M. and Bautz,E.K.F. (1987) *J. Mol. Biol.* **195**, 929–937.
20. Sweetser,D., Nonet,M. and Young,R.A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 1192–1196.
21. Kontermann,R., Sitzler,S., Seifarth,W., Petersen,G. and Bautz,E.K.F. (1989) *Mol. Gen. Genet.* **219**, 373–380.
22. Yano,R. and Nomura,M. (1991) *Mol. Cell. Biol.* **11**, 754–764.
23. James,P., Whelen,S. and Hall,B.D. (1991) *J. Biol. Chem.* **266**, 5616–5624.
24. Feinberg,A.P. and Vogelstein,B. (1983) *Anal. Biochem.* **132**, 6–13.
25. Feinberg,A.P. and Vogelstein,B. (1984) *Anal. Biochem.* **137**, 266–267.
26. Mémet,S., Saurin,W. and Sentenac,A. (1988) *J. Biol. Chem.* **263**, 10048–10051.
27. Baird,S.E. and Klobutcher,L.A. (1989) *Genes Dev.* **3**, 585–597.
28. Corden,J., Wasylyk,B., Buchwalder,A., Sassone-Coral,P., Kedinger,C. and Chambon,P. (1980) *Science* **209**, 1405–1414.
29. Furter-Graves,E.M. and Hall,B.D. (1990) *Mol. Gen. Genet.* **223**, 407–416.
30. Struhl,K. (1987) *Cell* **49**, 295–297.
31. Guarente,L. (1987) *Annu. Rev. Genet.* **21**, 425–452.
32. Struhl,K. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8419–8423.
33. Mahadevan,S. and Struhl,K. (1990) *Mol. Cell. Biol.* **10**, 4447–4455.
34. Brunk,C.F. and Sadler,L.A. (1990) *Nucleic Acids Res.* **18**, 323–329.
35. Leffers,H. Gropp,F. and Garrett,R. (1989) *J. Mol. Biol.* **206**, 1–17.
36. Spradling,A.C. (1981) *Cell* **27**, 193–201.
37. Osheim,Y.N. and Miller,O.L. (1983) *Cell* **33**, 543–553.
38. Delidakis,C. and Kafatos,F.C. (1989) *EMBO J.* **8**, 891–901.
39. Allison,L.A., Moyle,M., Shales,M. and Ingles,C.J. (1985) *Cell* **42**, 599–610.