Transcription of Yeast Mitochondrial Deoxyribonucleic Acid†

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ABSTRACT: The transcription of yeast mitochondrial DNA (mtDNA) was examined by analysis of the kinetics of hybridization of mitochondrial RNA (mtRNA) with highly labeled mtDNA. The RNA was purified by guanidium chloride–CsCl equilibrium centrifugation, and the DNA was labeled in vitro by nick translation. Hybridization levels at apparent saturation were consistently about 35%. After preannealing of the RNA, we found that approximately 30% hybridization remained. Hybridization levels were 10–15% at a R_0t (the product of RNA concentration and time) of 1 mol s L^{-1} and reached apparent saturation at R_0t values of 10^2–10^3 mol s L^{-1}. Hybridization with a restriction fragment containing sequences of 21S rRNA also reached an apparent saturation level of about 35%, but hybridization was elevated at low R_0t values (10^{-1} mol s L^{-1}). We also carried out hybridization with separated strands of cloned *Eco*RI fragment 7-pMB9 recombinant DNA molecules. One DNA strand hybridized to ≃42% and the other hybridized only to 2%. When a correction for pMB9 sequences was made, more than 95% of one mtDNA strand was transcribed. We conclude that there is extensive (at least 60%) transcription of one strand-equivalent of yeast mtDNA. The extent of transcription and the frequency distribution of total mtRNA populations were also evaluated in yeast grown anaerobically. The results could not be distinguished from those obtained in yeast grown under aerobic conditions. Finally, sequence differences between mitochondrial poly(A+) and poly(A−) RNA were investigated. The hybridization profiles were similar, except for the absence of an initial low level of hybridization at low R_0t values for poly(A+) RNA.

Although yeast mtDNA is 5 times larger than animal mtDNA (Borst, 1972), the same gene products have so far been demonstrated for mitochondrial genomes of both higher and lower eucaryotes (Schatz & Mason, 1974). The 10^7-dalton animal mitochondrial genome is completely accounted for by the known mitochondrial transcription and translation products, i.e., rRNA, tRNAs, and 8–10 mRNAs coding for some of the oligomeric peptides of the inner mitochondrial membrane. In contrast, 70–80% of the (5×10^7)-dalton yeast mitochondrial genome has no known information function. Bernardi et al. (1972) and Piperno et al. (1972) have shown that yeast mtDNA contains only 18% GC and that as much as 50% of the mitochondrial genome consists of interspersed high-AT (95%) stretches, 10^5–10^6 daltons in size. This finding has led to the suggestion that a large fraction of the yeast mtDNA represents high-AT spacer sequences. The relatively high GC content of yeast mitochondrial rRNA (Fauman et al., 1969; Reijnders et al., 1972) and tRNAs (Martin et al., 1976, 1977) supports this hypothesis.

It has not been established whether the high-AT stretches are transcribed. If they are, high-AU sequences may be removed during the processing of the primary transcript. On the other hand, high-AT mtDNA stretches may code for still unknown gene products which are not present in higher eucaryotes and the function of which may be related to regulation of phenomena such as oxygen adaptation and glucose repression; these functions are highly developed in yeast but not in animals.

The extent and nature of transcription of yeast mtDNA are still unclear; they appear to differ from the complete symmetrical transcription of HeLa cell mtDNA (Aloni & Attardi, 1971; Murphy et al., 1975). Transcripts equivalent in size to the mitochondrial genome have been demonstrated in HeLa cells (Aloni & Attardi, 1971), indicative of a single promoter site on each DNA strand. Prunell & Bernardi (1977), however, suggest multiple promoters for yeast mtDNA on the basis of the distribution of high-GC clusters demonstrated by digestion of mtDNA with several restriction endonucleases. Our laboratory (Lewin et al., 1977; Levins et al., 1979) has obtained evidence that there are separate promoters for each of the rRNA subunits; this argues against complete continuous transcription of yeast mtDNA. Double-stranded mtRNA, indicative of symmetrical transcription, has not been observed in yeast but has been found in HeLa cells (Young & Attardi, 1975). Previously, it was not technically possible to carry out experiments with separated mtDNA strands in yeast for evaluation of the presence or absence of symmetrical transcription.

The role of mtDNA transcription in the regulation of mitochondrial protein synthesis during anaerobiosis and glucose repression, conditions that markedly diminish the respiratory-enzyme content of the inner mitochondrial membrane, also has not been established. Mitochondrial transcription could be regulated directly by changes in the cellular environment, or it may respond secondarily to changes in nuclear transcription. Poyton & Kavanagh (1976) have shown that the mitochondrial synthesis of cytochrome oxidase peptides depends on the presence of peptides synthesized by cytoplasmic ribosomes. Therefore, cessation of nuclear transcription of mRNA for cytochrome oxidase would result in inhibition of mitochondrial translation, perhaps accompanied by secondary alteration of mitochondrial transcription.

In higher eucaryotes, mitochondrial mRNA has repeatedly been shown to contain poly(A) segments 60–100 nucleotides in length (Hirsch & Penman, 1973; Ojala & Attardi, 1974; Avadhani et al., 1974). In yeast, it has been difficult to demonstrate such segments (Groot et al., 1974). We have shown the existence of short poly(A) segments, 20–30 nucleotides in length, in yeast mtRNA by using poly(U)–Sepharose chromatography (Hendler et al., 1975). However, both poly(A+) and poly(A−) RNA stimulated labeled amino acid incorporation into protein by an *Escherichia coli* ribosomal system (Hendler et al., 1976). Multiple mtRNA species

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containing or lacking poly(A) segments have been noted in HeLa cells by Amalric et al. (1978), who used denaturing gel electrophoresis.

We investigated the extent of transcription of yeast mtDNA by analyzing the kinetics of hybridization of mtRNA with highly labeled mtDNA and DNA subfractions. Hybridization of mtRNA with total mtDNA, with a specific restriction fragment and with separated strands of another cloned restriction fragment, provides information about the magnitude and nature of mtDNA transcription. Experiments in which mtRNA is preannealed for removal of presumptive low-concentration symmetrical transcripts provide a minimal value for the transcription of a single DNA strand-equivalent. We also examined possible mitochondrial transcriptive differences in anaerobic and aerobic yeast, as well as sequence differences of mitochondrial poly(A+) and poly(A−) RNA.

Materials and Methods

Yeast Strains and Growth Conditions. Cells of the haploid strain of *Saccharomyces cerevisiae* D243-2B-R1 (Jakovcic et al., 1971) were grown aerobically and anaerobically (Rabinowitz et al., 1969) for at least eight cell divisions for 18–24 h to late-exponential phase and collected as described by Casey et al. (1972). The degree of anaerobiosis was established by measurements of the oxygen consumption of whole yeast (Rabinowitz et al., 1969) and by cytochrome *c* oxidase measurements of protoplast homogenates (Rabinowitz et al., 1969).

Preparation of mtRNA. Protoplasts and mitochondria were prepared according to the method of Grivell et al. (1971), as modified by Hendler et al. (1975). mtRNA was isolated from lysed mitochondria by phenol–chloroform–isoamyl alcohol extraction procedure (Hirsch & Penman, 1973). Analysis by 2.7% acrylamide gel electrophoresis (Hendler et al., 1975) showed 5–15% contamination by cytoplasmic RNA, as indicated by the ratio of cytoplasmic to mitochondrial rRNA. The DNA contamination of the RNA was removed by CsCl–guanidium chloride equilibrium centrifugation (Enea & Zinder, 1975). Fractions from the RNA peak were pooled, diluted, and alcohol-precipitated. The RNA pellet was dissolved in water and dialyzed against 0.23 M sodium chloride and 0.04 M sodium phosphate buffer (pH 6.8). No DNA was detected in the final RNA preparation (less than 0.02%) as determined by the DABA reaction (Kapp et al., 1974). The RNAs from three separate preparations of mitochondria grown anaerobically were pooled and used for hybridization.

Preparation and in Vitro Labeling of mtDNA. mtDNA was isolated from mitochondria (Casey et al., 1974) and purified by two CsCl density gradient centrifugations. No nDNA contamination was detected by isopyknic centrifugation in the analytic ultracentrifuge. In vitro labeling of mtDNA was obtained by nick translation with *E. coli* polymerase I (P-L Biochemicals, Inc., Milwaukee, WI) incubated for 1 h at 18 °C (Monoyama & Pagano, 1973). The specific activity of the DNA obtained varied from 3×10^6 to 10×10^6 cpm/μg of DNA. The size of the DNA as determined by alkaline sucrose gradient analysis (Studier, 1965) corresponded to a molecular weight of $(2–3) \times 10^5$. The DNA was heat-denatured, and the single-stranded DNA was absorbed and eluted from a hydroxyapatite column for removal of "zero-time" DNA (Jakovcic et al., 1975). The DNA was then concentrated with butanol (Stafford & Bieber, 1975) and dialyzed against 0.23 M sodium chloride and 0.04 M sodium phosphate buffer (pH 6.8).

Hybridization of mtRNA to [^{3}H]mtDNA. Hybridization of a vast excess of mtRNA to mtDNA was carried out in solution at 60 °C in 0.23 M sodium chloride and 0.04 M phosphate buffer (pH 6.8) either for various periods of time or at different concentrations of RNA. RNA–DNA hybrids were detected with *Neurospora* single-stranded nuclease purified according to the method of Rabin et al. (1971) and stored at −70 °C in 50% glycerol. The 100-μL assay system contained $(1–2) \times 10^3$ cpm of mtDNA, 1 μg of unlabeled native DNA, 0.1 μg of denatured nDNA obtained from a ρ^0 petite, 0.05 M Tris, 0.005 M $MgCl_2$, 0.06 M NaCl, and 0.01 M $NaPO_4$ (pH 8.2). With the addition of unlabeled native DNA in the assay mixture, there was less than 3% digestion of double-stranded DNA, while digestion of single-stranded DNA was >95% complete. With each set of hybridizations, a zero-time control (complete reaction nonincubated) and a control incubated without RNA were run. Zero-time controls were consistently about 5%; incubated DNA controls ranged between 5 and 15% and varied with the duration of the reaction and the specific activity of the DNA. To ensure that the RNA preparations did not contain significant amounts of DNA, we annealed the hybridization reactions to specified R_0t values, diluted them to 0.012 M NaCl and 0.04 M phosphate buffer (pH 6.8), and incubated them with 10 μg/mL RNase for 16 h at 37 °C (Galau et al., 1974). Hybridization values agreed closely (within 3%) with those obtained with DNA controls incubated for the same time intervals. Control values were subtracted from the hybridization values. The hybridization results are expressed as a function of the product of RNA concentration (R_0) and the time of incubation (t) in moles of nucleotide seconds liter^{-1}. Analysis of the RNA by 2.7% polyacrylamide electrophoresis before and after hybridization showed similar patterns indicating no substantial degradation had occurred.

Purification of Restriction Fragment Hpa 6. Restriction fragment *Hpa* 6 was isolated from *Hpa* I limit digests of mtDNA from strain MH41-7B as described by Morimoto et al. (1977). The purity of the isolated fragment was demonstrated by electrophoresis on a 1% agarose gel. No degradation or contamination from other fragments was detected.

Strand Separation of Cloned Mitochondrial EcoRI Fragment 7-pMB9 DNA. Strand separation of cloned mitochondrial *Eco*RI fragment 7-pMB9 DNA (Berg et al., 1979) was carried out by a modification of the method of Szybalski et al. (1968) and Hayward (1972). The recombinant DNA was labeled in vivo with [^{3}H]thymidine to a specific activity of 1.1×10^6 cpm of DNA. The covalently closed circular DNA was isolated from cleared lysate (Guerry et al., 1973) by CsCl–ethidium bromide centrifugation (Radloff et al., 1967). The circular DNA was digested with *Bam* H1 endonuclease (BRL, Bethesda, MD), which recognizes a single site on the recombinant molecule. Incubation was carried out at 37 °C for 30 min in 100 mM Tris, pH 7.4, 10 mM $MgCl_2$, and 10 μg/mL bovine serum albumin. The complementary strands of the linear DNA were then separated by complexing with the synthetic ribopolymer poly(UG) at a weight ratio of poly(UG)/DNA of 1:1 in a solution containing 0.01 volume of 10% $NaDodSO_4$ and 1 mM EDTA, pH 8. The solution was heated at 95 °C for 3 min, quenched in ice-cold H_2O, and incubated first at 4 °C for 50 min and then at room temperature for 20 min. One-tenth volume of 60% sucrose–0.05% bromphenol blue was added, and samples containing 6 μg of DNA in 50 μL were layered onto 1.4% agarose slab gels (1 × 30 cm). Electrophoresis was carried out for 24 h at room temperature at 80 V in 20 mM Tris, 18 mM NaH_2PO_4, and 0.5 mM EDTA, pH 7.5. The separated strands were visualized by fluorescence after staining with ethidium bromide (1

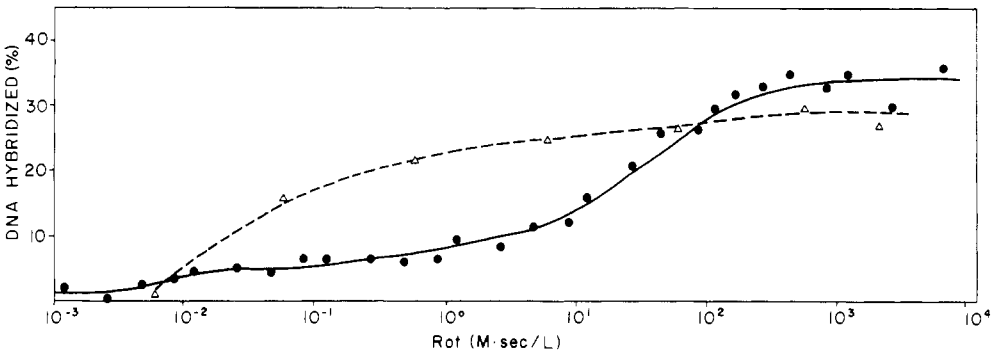

FIGURE 1: Hybridization of yeast [^{3}H]mtDNA and *Hpa* I fragment 6 with mtRNA. Purified yeast mtDNA (●) or *Hpa* I fragment 6 (△), labeled to a specific radioactivity of 1×10^7 cpm/μg of DNA by nick translation with *E. coli* DNA polymerase I, was hybridized with mtRNA purified by CsCl–guanidium chloride centrifugation as described under Materials and Methods. Hybridization was measured with *Neurospora* single strand specific nuclease. Nonincubated zero-time controls averaged 5% hybridization, whereas incubated [^{3}H]mtDNA controls and hybridization mixtures digested with RNase at low ionic strength varied between 5% for 1–2 h of incubation and 10–15% for 24–48 h of incubation. Similar results were obtained with four different RNA preparations and two different mtDNA preparations.

μg/mL) in the above buffer for 30 min at room temperature. The bands were excised and the DNA was extracted from the gel slices by a freeze–squeeze method (Thuring et al., 1975). Carrier single-stranded salmon sperm DNA, 100 μg/μg of cloned DNA, was added in 0.24 M sodium phosphate buffer, pH 6.8. The DNA was sheared by sonication at maximal speed at 5 °C at 30-s intervals. To remove the possibility of contamination by the opposite strand, we incubated each band at 60 °C for 16 h and removed the duplex DNA by hydroxyapatite chromatography. This procedure was repeated a second time for the lower migrating band (band II). The single-stranded DNA was concentrated in dialysis bags with Sephadex G-50 and dialyzed in hybridization buffer. Hybridization was carried out with an excess of total mtRNA in 0.23 M NaCl and 0.04 M sodium phosphate, pH 6.8, for 16 h at 60 °C. The extent of [^{3}H]DNA annealing was assayed by measurements of resistance to S_1 nuclease from *Aspergillus oryzae* (Miles Biochemical Laboratories, Elkhart, IN). The reaction mixture containing 0.3 M NaCl, 0.05 M sodium acetate, pH 4.5, 0.001 M $ZnSO_4$, and 0.5 μL of enzyme per μg of DNA was incubated at 37 °C for 30 min.

Ratio of Radioactivity in the Plasmid Vector pMB9 to the Inserted EcoRI Fragment 7. The [^{3}H]thymidine-labeled recombinant DNA was digested with *Eco*RI endonuclease (Morimoto et al., 1977), and the vector was separated from the insert by 1% agarose gel slab electrophoresis (0.6 × 16.5 cm). The two bands were excised, placed in the counting vials containing 1 mL of concentrated NCS (Amersham/Searle Corp., Arlington Heights, IL), and incubated overnight at room temperature. Ten milliliters of toluene was then added, and samples were counted in a Packard liquid scintillation counter.

Results

Transcription of Total Yeast mtDNA and *mtDNA Fractions.* We evaluated the extent of mtDNA transcription by examining the hybridization of mtRNA with purified mtDNA that was labeled to high specific activity by nick translation. The RNA was purified from DNA by guanidium chloride–CsCl equilibrium centrifugation. That this procedure removed the last traces of DNA was demonstrated by cocentrifugation of [^{3}H]DNA with the mtRNA. Guanidium chloride–CsCl centrifugation was more effective than hydroxyapatite chromatography or treatment with DNase in eliminating DNA contamination. Hybridization was analyzed by resistance to single strand specific *Neurospora* nuclease. Figure 1 illustrates the hybridization of total mtDNA with mtRNA. The data are plotted as percent DNA hybridized vs. R_0t, the product of RNA concentration (R_0) and time of incubation (t). An apparent hybridization plateau (at 4–5%) for a R_0t of about 10^{-1} mol s L^{-1} may represent hybridization of high-concentration rRNA and tRNA transcripts, and the increment to 12% hybridization at a R_0t of about 10^0–10^1 mol s L^{-1} may represent mRNAs. The higher levels of hybridization (30–40%) at very high inputs of RNA may be due to large transcripts which are being processed to smaller functional units. Alternatively, they could represent low-concentration mRNAs.

Table I: Effect of Preannealing of RNA on Mitochondrial RNA-DNA Hybridization[a]

pretreatment of RNA	% DNA hybridized
none	35.2 ± 1.4
preanneal	29.2 ± 2.6

[a] RNA-DNA hybridization was done at $R_0t = 1.2 \times 10^3$ mol s L^{-1} under standard conditions. The RNA was annealed to $R_0t = 2 \times 10^3$ mol s L^{-1}. Data are expressed as the mean ± standard deviation ($n = 4$).

In agreement with the interpretation that the early plateau on the R_0t curve of the total mtDNA represents rRNA sequences are the results of the hybridization of *Hpa* I restriction fragment 6 with mtRNA. This fragment is almost completely occupied by sequences coding for 21S rRNA (Morimoto et al., 1978), and it has a GC content of 28% compared to the GC content of 18% for total yeast mtDNA (Morimoto and Rabinowitz, unpublished experiments). The R_0t curve of *Hpa* I restriction fragment 6 rises rapidly at low RNA imputs ($R_0t = 6 \times 10^{-2}$ mol s L^{-1}), plateaus early, and reaches a hybridization value similar to that for total mtDNA (Figure 1).

Effect of Preannealing of RNA on Mitochondrial RNA–DNA Hybridization. Since symmetrical transcription has been described in HeLa cell mtDNA (Murphy et al., 1975), we sought to evaluate its possible effects on our measurements of the extent of transcription of yeast mtDNA. To remove presumptive low-concentration symmetrical transcripts, we preannealed the RNA prior to its hybridization with DNA (Table I). Hybridization values were reduced by about 6% (from 35 to 29%) by this procedure. The results are consistent with the presence of symmetrical transcription but may be due instead to secondary structural changes of mtRNA which may alter the hybridization values. It should be noted that 30% hybridization still remains after preannealing of the RNA. One can therefore conclude that at least double this amount, i.e., 60% of one mtDNA strand-equivalent, is being transcribed.

Table II: Hybridization of *Eco*RI Fragment 7-pMB9 DNA with mtRNA[a]

DNA strand	hybridization (%) control (no RNA)	+mtRNA	−control	ratio of [^{3}H]pMB9/ mtDNA	hybridization (%) corrected for pMB9 sequences
I	1.3	43.1	41.8	1.30 ± 0.15 SD, $n = 12$	96.5
II	4.9	7.6	2.3		5.3

[a] Strands of *Eco*RI fragment 7-pMB9 [^{3}H]DNA were separated by agarose gel electrophoresis as described under Materials and Methods. Hybridization was carried out at a R_0t of 10^3 mol s L^{-1}, and hybridization was analyzed with S_1 nuclease. The ratio of [^{3}H]thymidine in pMB9 to that in mtDNA *Eco*RI fragment 7 was measured as described under Materials and Methods and used for estimates of the hybridization of inserted mtDNA. Hybridization results represent an average of two separate experiments, each performed in duplicate.

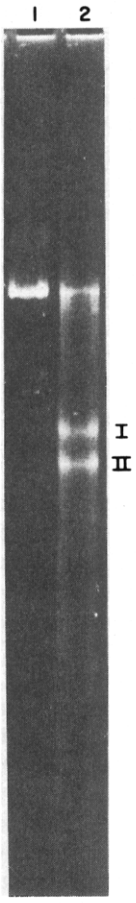

FIGURE 2: Strand separation of cloned mitochondrial *Eco*RI fragment 7-pMB9 DNA. The circular DNA was digested with *Bam* H1 endonuclease, heat-denatured, and complexed with poly(UG), and the strands were separated by 1.4% agarose slab gel electrophoresis as described under Materials and Methods. Slot 1, *Bam* H1 digest; slot 2, separated strands (bands I and II).

Hybridization of Separated Strands of EcoRI Fragment 7-pMB9 DNA with mtRNA. In order to gain more information about the nature of the transcription of yeast mtDNA, we examined the hybridization of separated strands of *Eco*RI fragment 7-pMB9 DNA. *Eco*RI restriction fragment 7 probably contains the gene for one of the peptides of the oligomycin-sensitive ATPase (R. Morimoto, personal communication). Although strand separation of total yeast mtDNA, or of individual restriction fragments, has not yet been achieved, strand separation of the recombinant molecule is facilitated by the considerable strand bias of the plasmid vector pMB9. DNA was labeled in vivo with [^{3}H]thymidine, and the circular recombinant molecule was isolated and digested with *Bam* H1 restriction endonuclease. After addition of poly(UG) and denaturation, the strands were separated by agarose gel electrophoresis (Figure 2). Each band was excised, eluted, and hybridized with itself to remove residual contamination of the opposite strand. The upper DNA band (strand I) hybridized with mtRNA to a level of 42% at a R_0t of 10^3 mol s L^{-1}, whereas the lower band (strand II) hybridized to a level of only 2% (Table II).

For the calculation of the percent transcription of mtDNA restriction fragment 7, the [^{3}H]thymidine-labeled recombinant DNA was digested with *Eco*RI nuclease, and the radioactivity in the pMB9 and *Eco*RI fragment 7 bands was measured. The ratio of the radioactivity in the vector to that in the insert was 1.3 ± 0.15 SD. This ratio is consistent with the fact that the insert, although considerably smaller than the vector, has a much higher A-T content. When corrected for the presence of the pMB9 sequences, the hybridization level of the upper mtDNA band was about 95%, whereas that of the lower band was only about 5%.

Hybridization of mtDNA with RNA Isolated from Anaerobically and Aerobically Grown Yeast. Anaerobically grown yeast has a markedly reduced respiratory enzyme activity. To determine whether or not these changes are due to altered mtDNA transcription, we compared the kinetics of hybridization of mtDNA with mtRNA isolated from anaerobically and aerobically grown yeast. The cytochrome oxidase activity of anaerobic yeast protoplast homogenates was 0.29

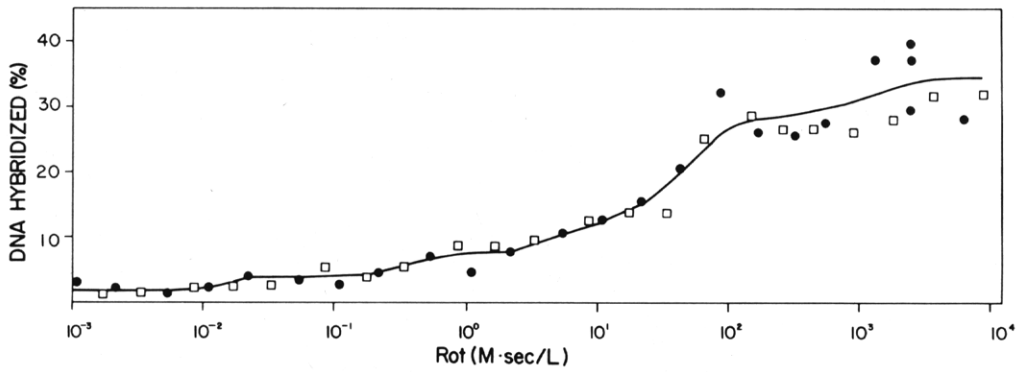

FIGURE 3: Hybridization of [^{3}H]mtDNA with mtRNA isolated from aerobically (●) and anaerobically (□) grown yeast. The specific activity of [^{3}H]mtDNA was 1×10^7 cpm/μg of DNA.

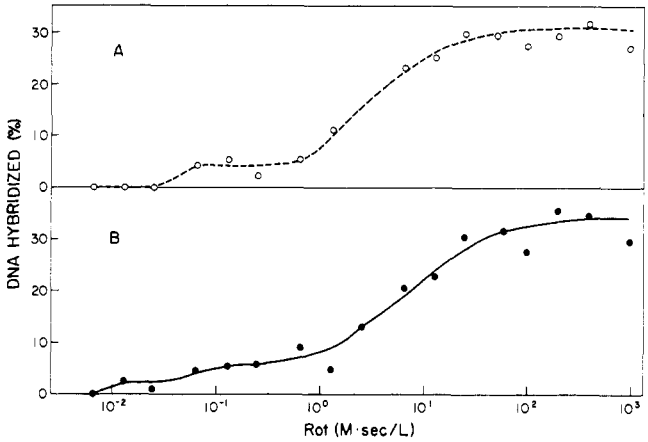

FIGURE 4: Hybridization of mitochondrial poly(A+) RNA and poly(A−) RNA to mtDNA. Poly(A+) RNA (○) was separated from poly(A−) RNA (●) with poly(U)–Sepharose as previously described (Hendler et al., 1975). Hybridization was carried out with mtDNA of specific radioactivity of 1×10^7 cpm/μg of DNA.

(kat/min)/mg of protein, compared to 7.8 (kat/min)/mg of protein in aerobic yeast. The hybridization saturation plateaus reached with RNA isolated from aerobically or anaerobically grown yeast were similar (Figure 3), and the R_0t curves could not be differentiated from each other.

Hybridization of Poly(A+) RNA with mtDNA. We have previously reported on the isolation, by poly(U)–Sepharose chromatography, of a yeast mtRNA fraction that contains short segments of poly(A) (20–30 nucleotides) (Hendler et al., 1975). Both poly(A+) and poly(A−) RNA stimulated protein synthesis in *E. coli* cell-free systems (Hendler et al., 1976). We therefore investigated whether the poly(A+) RNA had sequences that were not present in the poly(A−) RNA. Hybridization of poly(A+) RNA is compared with that of poly(A−) RNA in Figure 4. The R_0t curves have similar configurations, and both have plateaus of approximately 30–35%, except that the level of hybridization of poly(A+) RNA at a R_0t of about 10^{-2} mol s L^{-1} is lower. This difference could represent removal of rRNA. We conclude that most of the RNA sequences may be shared by poly(A+) and poly(A−) RNA.

Discussion

Known and presumptive transcripts account for only about 15% of the sequences of yeast mtDNA. The data presented here, however, indicate that a considerably greater fraction of yeast mtDNA is transcribed. Hybridization levels of 30–40% were obtained at high RNA inputs in numerous experiments, and 30% hybridization was noted after extensive preannealing of mtRNA, which would be expected to remove low-concentration symmetrical transcripts. Thus, there appears to be a minimum transcription of 60% of one single strand-equivalent of yeast mtDNA. Very likely, a still larger fraction of mtDNA is transcribed. Our hybridization system may slightly underestimate saturation levels, as suggested by the 30–35% hybridization between mtRNA and the restriction fragment *Hpa* 6, which, as we have shown, consists almost completely of rRNA sequences (Morimoto et al., 1978). Therefore, this fragment should have reached a hybridization level near 50%. The R_0t curves obtained with this restriction fragment show considerable hybridization at low R_0t values, as would be expected for rDNA. The gradual increase in hybridization at higher R_0t values is perhaps explained by the presence of a 1.2-kbp intervening sequence within the 21S rRNA gene (Borst et al., 1977; Jacq et al., 1977; Bos et al., 1978). The RNA sequences presumably removed by splicing would be expected to be present in relatively low concentrations and anneal only at high R_0t values.

The hybridization of separated *Eco*RI fragment 7-pMB9 strands supports the proposal that a large fraction, if not all, of a single strand-equivalent of mtDNA is transcribed. More than 95% of one strand of this restriction fragment was transcribed, whereas only 5% hybridization of the opposite strand was observed. The latter value may represent residual contamination with band 1. *Eco*RI fragment 7 has a molecular size of 1.3×10^6 daltons, which represents only 2% of the yeast mitochondrial genome. Its molecular organization, however, may be similar to that of total mtDNA, i.e., it may be composed of high-AT sequences interspersed between relatively high-GC sequences.

The extremely high level of hybridization of strand I of *Eco*RI fragment 7, together with the substantial level of hybridization observed for total mtDNA, suggests that the high-AT presumptive spacer sequences are transcribed. Such an interpretation is consistent with the observation that many yeast mtRNA species, which together have an aggregate molecular weight of 47% (Van Ommen & Groot, 1977) to more than 100% (Levins et al., 1979) of a single strand-equivalent of yeast mtDNA, are detected by polyacrylamide–agarose gel electrophoresis. Some of the high molecular weight transcripts could represent precursors of functional transcripts. If the high-AT sequences are transcribed, subsequent processing of the primary transcript into functional transcripts probably results in the excision and removal of AU-rich sequences.

In HeLa cells, complete symmetrical transcription of mtDNA has been established (Aloni & Attardi, 1971; Murphy et al., 1975). The possibility of symmetrical transcription of yeast mtDNA is not eliminated by our data. The extremely low hybridization of strand II of *Eco*RI fragment 7 and the hybridization levels, with total mtDNA, of less than 50% do not rule out symmetrical transcription, since in the steady state low-concentration symmetrical transcripts might preferentially hybridize with their high-concentration RNA complements and therefore be unavailable for hybridization with mtDNA. Our results, however, are consistent with extensive asymmetric transcription of yeast mtDNA. This possibility is supported by our observation of asymmetrical transcription in vitro by a transcription complex of yeast mtDNA and mtRNA polymerase (Lewin et al., 1977; Levins et al., 1979).

We have also compared the hybridization of yeast mtRNA isolated from anaerobically and aerobically grown yeast with mtDNA but could detect no difference in the kinetics of hybridization or in the hybridization saturation level. The lower saturation levels with mtRNA from anaerobic yeast, which we obtained in our previous preliminary study (Hendler et al., 1976), were probably due to the relatively low R_0t values achieved. If mitochondrial translation of oligomeric peptides is controlled by cytoplasmic peptides, as suggested by Poyton & Kavanagh (1976), the turning off of mitochondrial protein synthesis in anaerobiosis may be independent of mitochondrial transcription.

We also could find no difference between the kinetics of hybridization with mtDNA of poly(A+) RNA and that of poly(A−) RNA, except perhaps for the removal of rRNA in the former. It appears that yeast mitochondrial mRNA species are present in forms that do and those that do not contain poly(A).

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pMB9 DNA. We also acknowledge the excellent technical assistance of Epifania Rulloda.

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