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MITOCHONDRIAL rRNA-CONTAINING PETITE STRAINS OF YEAST (SACCHAROMYCES CEREVISIAE) SHOW A NORMAL NUCLEAR-MITOCHONDRIAL STRINGENT RESPONSE

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The nuclear-mitochondrial stringent response was examined in isonuclear ρ^+, 21S rRNA-containing ρ^-, and ρ^0 strains of S. cerevisiae. By 30 min after nutritional downshift, nuclear rDNA transcription falls to 15% of control levels congruently in all strains, as assayed via whole-cell RNA or by hybrid selection of specific double-labeled transcripts. Both in vivo and in vitro, the mitochondrial stringent response is identical between the ρ^- strain and its parental ρ^+ strain, and in both, the kinetics and magnitude of the organellar response mirror those of the nuclear response. The data show that mitochondrial transcription and protein synthesis are not required for stringent regulation of either nuclear or mitochondrial rDNA transcription.

It is well known that for many strains of bacteria, perturbations in growth conditions result in coordinate and selective cessation of transcription of both rRNA and r-protein genes (1-3); general mRNA populations are initially much less affected by the perturbation. This so-called stringent response has been observed in eukaryotes as well, and it has been most extensively studied in yeast. As in bacteria, starvation of yeast for an essential amino acid leads to coordinated curtailment of r-protein gene and rDNA transcription, while the bulk of cellular mRNA and tRNA is less affected (4-7). In addition to amino acid starvation, a stringent response can be induced in yeast by nutritional shiftdown or mild temperature shock (8-9), but in all cases the transcriptional response is selective for genes specifying ribosome components.

The stringent response extends to transcription of mt rDNA in yeast. In one set of studies, tyrosine starvation and nutritional shiftdown were shown to result in a 5-7 fold decrease in the rates of both mt and nuclear rDNA transcription, compared to those in non-manipulated cells (9, 10); some strain-

ABBREVIATIONS USED
mt, mitochondrial; ts, temperature sensitive; r-protein, ribosomal protein; mt r-protein, mitochondrial ribosomal protein; SSC, 3 M sodium citrate-0.3 M NaCl; TCA, trichloroacetic acid.

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dependent variation was seen in these studies. Treatment of cells with cycloheximide induced a stringent response in both nuclear and mt genomes, but inhibition of mt protein synthesis by chloramphenicol did not affect production of nuclear or mt rRNA; two ts mutant strains of yeast with different lesions in the cytoplasmic protein synthetic system also showed congruent nuclear-mt stringent responses after shift to the nonpermissive temperature (10).

Recent studies have shown that the quality and/or quantity of mt DNA in yeast cells (i.e., being ρ^- or ρ^o) can influence transcription of portions of the nuclear rDNA repeats in some nuclear backgrounds (11, 12; see also Discussion). This suggested to us that the overall condition of the mt genome might have an effect on transcription of nuclear and/or mt rDNA sequences during a stringent response. We report here that both ρ^- and ρ^o strains of yeast show a nuclear stringent response identical to that of an isonuclear ρ^+ strain, when subjected to nutritional shiftdown. We show that in mt rRNA-containing ρ^- strains the nuclear-mt stringent response is fully intact, and we show that the mt portion of the stringent response can be reproduced from ρ^+ and mt rRNA-containing ρ^- strains in an in vitro mt transcription assay.

METHODS

Yeast strains, media, cell growth. S. cerevisiae strains used were: IL8-8C (MATα, his1, trp1), a ρ^+ strain (13); F11 (MATα, his1, trp1), a mt 21S rRNA-containing ρ^- strain derived from IL8-8C (13-16); and IL8/0 (MATα, his1, trp1), a ρ^o strain independently derived from IL8-8C (17). Growth of all strains was at 30°C in a rotary shaking water bath at 250 rpm. Growth medium for all experiments was modified minimal medium (18), supplemented with 20 mg/L of the appropriate amino acids (19); carbon source was 2% glucose. Media used for nutritional shiftdown manipulations were 1X minimal medium, and the same medium diluted to a concentration of 0.05X with sterile water (9).
Nutritional shiftdown, labeling of cells. Cells were grown for 16 hrs in 1X medium containing 2 μCi/ml ^{14}C uracil so as to uniformly label RNA; 10 μg/ml unlabeled uridine was included to insure linear uptake of labeled precursor (9, 10). At early log phase growth (A_{600}=0.4-0.5) cultures were divided in half, and each half was harvested by centrifugation and washed. One half was resuspended in fresh 1X medium and the other in 0.05X medium, each containing unlabeled uridine. The cultures were then allowed to grow normally for 30 min, whereupon ^3H-uridine was added to each to 30 μCi/ml final concentration.
Preparation of RNA, hybrid selection of transcripts. At various times after ^3H-uridine addition, aliquots of cultures were harvested, and whole-cell RNA was prepared from the unspheroplasted cells as described (20). For some studies, the purified double labeled RNA was counted directly. In others, specific nuclear and mt transcripts were isolated from the labeled whole cell mixtures via hybrid selection. The hybrid selection procedure employed plasmid clones of nuclear and mt genes (Legend, Figure 2) which had been alkali-denatured and bound to ZetaProbe membranes; selection of specific transcripts was done at 42°C in a buffer containing 40% formamide, 5X SSC, 10 mM $NaPO_4$ (pH 7.5), 2X Denhardts solution, and 0.1% sodium dodecylsulfate (see 21). Various control experiments using this hybrid selection procedure showed a rigorous selection of specific nuclear and mt transcripts in all cases (not shown).
Preparation of mitochondria, in vitro mt transcription assay. In all experiments, cells were spheroplasted (19, 22), regrown for 3 hr at 30°C with shaking in isotonically adjusted 1X medium as described (9), and reharvested.

One half of the regrown culture was resuspended in isotonically adjusted 1X medium, and the other in isotonically adjusted 0.05X medium; both were allowed to grow at 30°C with shaking for 1 hr, whereupon cells were harvested and mitochondria were prepared from each half (9, 22). The in vitro mt transcription assay was done as originally described (23; see also Legend, Figure 3), and incorporation of [α-^{32}P]UTP into mt RNA was monitored via TCA precipitation onto glass fiber filters. Control experiments using mitochondria from both strains F11 and IL8-8C showed that incorporation of UT^{32}P into mt RNA is linear for at least 40 min, and that incorporation of radioactive precursor is sensitive to 5 μg/ml ethidium bromide, as would be expected if the RNA being synthesized is mt in origin (23; data not shown).

RESULTS

In eukaryotic cells, transcripts from the highly repeated nuclear rRNA genes always constitute a large proportion of whole cell RNA. Because a stringent response powerfully affects nuclear rDNA transcription, it is possible to assess the overall cellular level of such a response by monitoring incorporation of labeled precursors into whole cell RNA over time, after the imposition of stringent conditions. Fig. 1A shows one typical such experiment, in which the rate of incorporation of ^{3}H-uridine into newly synthesized whole-cell RNA in downshifted cells of strain IL8-8C is 7-fold lower than that in non-downshifted cells; these data are consistent with results from earlier, similar studies using other yeast strains (7, 9). Figs. 1B-1C show similar experiments suggesting that the overall stringent response in ρ^- strain F11 and ρ^o strain IL8/0 are identical to that of parental strain IL8-8C, when assayed by this method. These data indicate that the stringent response in all strains is fully in effect at the earliest point assayed after shiftdown in the experiments. Similar experiments with a 16S mt rRNA-containing ρ^- strain not derived from IL8-8C gave results identical to those in Fig. 1 (not shown).

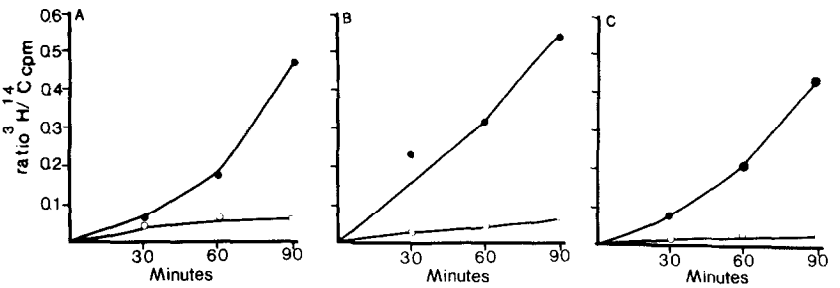

Figure 1. Stringent response at the whole cell RNA level in isonuclear strains of yeast. Panel A, ρ^+ strain IL8-8C; Panel B, 21S-rRNA containing ρ^- strain F11; Panel C, ρ^o strain IL8/0. As described in Methods, cells were uniformly labeled with ^{14}C-uracil; half of each culture was then shifted from 1X to 0.05X medium, and newly synthesized RNA was labeled with ^{3}H-uridine in both halves. At various times after shiftdown and addition of ^{3}H-uridine, RNA was prepared from both cultures and counted. Symbols are: ●, whole cell RNA from cells in 1X medium; ○, whole cell RNA from cells shifted from 1X to 0.05X medium.

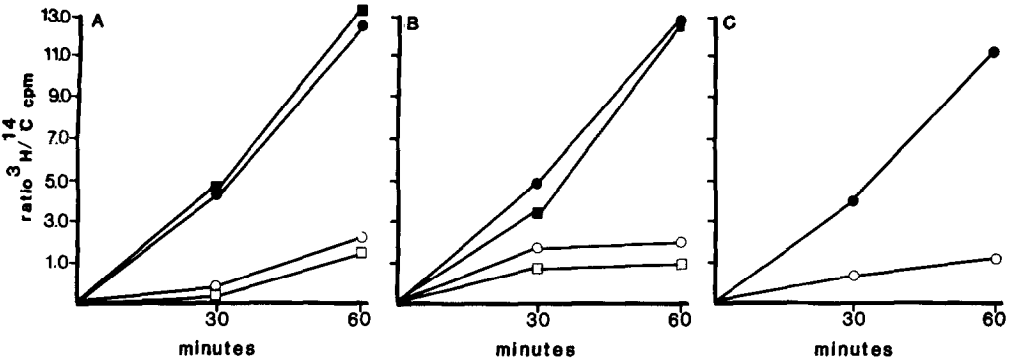

Figure 2. Stringent response in isonuclear strains at the level of specific nuclear and mt rDNA transcripts. Panel A, ρ^+ strain IL8-8C; Panel B, 21S rRNA-containing ρ^- strain F11; Panel C, ρ^o strain IL8/0. Growth, labeling of cells, and preparation of RNA were as in Fig. 1; specific transcripts were isolated from whole cell RNA mixtures by hybrid selection, using plasmid clones for nuclear 18S+26S rRNA genes (pJHC310) and the mt 21S rRNA gene (pYmA1248-2e). Symbols are: ●, 18S+26S rRNA from cells in 1X medium; ○, 18S+26S rRNA from cells shifted from 1X to 0.05X medium; ■, 21S mt rRNA from cells in 1X medium; □, 21S mt rRNA from cells shifted from 1X to 0.05X medium.

In order to assess more precisely the effect of stringent conditions on transcription of individual nuclear and mt genes, we repeated the experiments in Fig. 1 but isolated specific double labeled transcripts from the nuclear 18S+26S and mt 21S rRNA genes via a hybrid selection procedure. Figs. 2A-2C confirm that in strains IL8-8C, F11, and IL8/0, the rate of transcription of nuclear rRNA genes has fallen to about 15% of control values by 30 min after the start of ^{3}H-uridine labeling. Figs. 2A-2B further show that the level and kinetics of stringent curtailment in mt rDNA transcription are identical between ρ^+ strain IL8-8C and its 21S rRNA-containing ρ^- derivative. The mt portion of the stringent response in these experiments mirrors closely that for nuclear rRNA genes, both in the ρ^+ strain and in its ρ^- derivative. Strain IL8/0 has, of course, no mtDNA and thus lacks all mt transcription. Similar experiments using the 16S rRNA-containing ρ^- strain gave results congruent to those for strain F11 (not shown). The data show that neither mt transcription nor mt protein synthesis is required for regulation of either the nuclear or mt stringent response. The similarity between nuclear and mt responses seen in these experiments may suggest that stringent curtailment of rDNA transcription in both genomes is coordinated by some unknown mechanism (see Discussion).

Transcription and processing for the mt 21S gene in ρ^- strain F11 is known to be essentially identical to that in its parental strain (14-16); strain F11 is also known to assemble the large subunit of the mt ribosome, even though mt protein synthesis cannot take place (24). Further, transcription and processing for the 21S gene have been shown to take place in an *in vitro* mt assay system (23, 25). Fig. 3 shows that the mt portion of the stringent

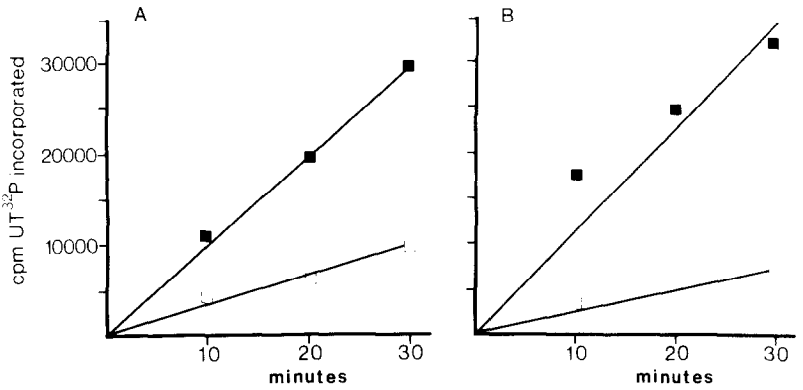

Figure 3. Stringent response in purified mitochondria derived from ρ^+ strain IL8-8C (Panel A) and ρ^- strain F11 (Panel B). As described in Methods, mitochondria were prepared from regrown spheroplasts which had remained in 1X medium or which had been shifted from 1X to 0.05X medium after regrowth. The organelles were used in an in vitro mt transcription system to determine the relative rates of incorporation of $UT^{32}P$ into mt RNA; the suspension of mitochondria added to each assay was adjusted such that each tube received 5 mg mt protein. Symbols are: ■, incorporation in mitochondria derived from 1X medium-grown spheroplasts; □, incorporation in mitochondria derived from downshifted spheroplasts.

response in strains IL8-8C and F11 can be reproduced in the same in vitro mt transcription system. In our hands, mitochondria prepared from regrown spheroplasts subjected to nutritional downshift show a 2-4 fold lower rate of incorporation of $UT^{32}P$ into mtRNA than do organelles derived from regrown, non-downshifted spheroplasts. Experiments similar to that in Fig. 3B using the 16S rRNA-containing ρ^- strain gave results identical to those for strain F11 (not shown). The magnitude of mt transcriptional curtailment in such in vitro assays is less than that seen in vivo; experiments not given here suggest that were spheroplasts to be regrown for long periods prior to downshift, the level of the in vitro response would approach that seen in vivo. Regardless of the magnitude of the response in vitro, these data confirm that the mt stringent response in strain F11 is identical to that of its parental ρ^+ strain, and that the response is fully intact in the 21S rRNA-containing ρ^- strain.

DISCUSSION

Recent results from another laboratory show that in certain nuclear backgrounds, transcription of specific nuclear DNA sequences in yeast can be affected by the overall respiratory competance of the cell and/or the condition of its mt genome (11, 12). Importantly, this study showed that transcripts from the supposedly nontranscribed spacer region of nuclear rDNA repeats are increased many-fold in some ρ^0 strains, and increased somewhat in certain ρ^- strains, compared to levels in related ρ^+ cells; this effect was correlated

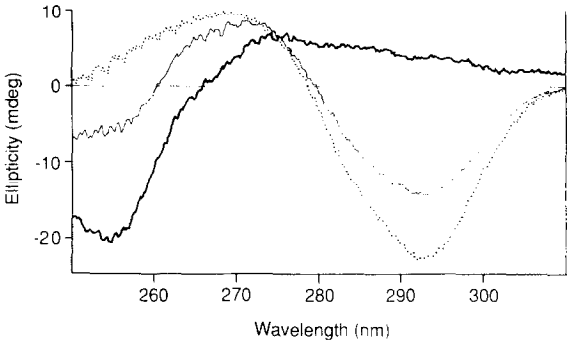

Fig. 3: CD spectra of poly[d(G-m^{5}C)] in aqueous ethanol solutions. Solvent also contained 5 mM Tris·HCl, 50 mM NaCl, pH 7.0. Ethanol concentrations (M) were 2.9 (——), 3.1 (– – –), and 3.2 (·····). All spectra are expanded on the ellipticity scale by a factor of 3.0.

The B-to-Z transition has previously been observed in the presence of organic cosolvents such as dimethylsulfoxide, ethanol, ethylene glycol, formamide, glycerol, and trifluoroethanol (1,5,14,16,17; B. G. Rowan and R. S. Preisler, unpublished results). The effect of cosolvent hydrophobicity was tested by comparing the water-miscible alcohols. Sample CD spectra of poly[d(G-m^{5}C)] in aqueous ethanol solutions (Figure 3) show spectral changes and cooperativity similar to those of the salt-induced B-to-Z transition (Figure 1). Previous reports (5,14,16) gave similar transition midpoint ethanol concentrations for both poly[d(G-C)] and poly[d(G-m^{5}C)]. Effectiveness in driving the transition of poly[d(G-m^{5}C)] increased with alkyl group size, from methanol (one carbon) to 2-methyl-2-propanol (four carbons--Table 3). The beginning of a similar trend was observed in poly[d(G-C)], in that ethanol was more effective than methanol (Table 3). With the longer chain alcohols lower concentrations (below 5-6M) favored the B-DNA conformer of poly[d(G-C)]. Higher concentrations produced, instead of a B-to-Z transition, anomalous CD spectra (results not shown), perhaps indicating denaturation or aggregation of the duplex.

TABLE 3. B-to-Z transition midpoint concentrations in aqueous alcohol solutions

Alcohol	poly[d(G-C)]	poly[d(G-m^{5}C)]
CH_3OH	13	4.6
CH_3CH_2OH	8.2	3.1
$CH_3CH_2CH_2OH$	---	2.6
$CH_3CHOHCH_3$	---	2.5
$(CH_3)_3COH$	---	2.2

Alcohol concentrations are in M. Conditions are as described in Materials and Methods and in Figure 3.

single regulatory factor, but rather by different factors whose synthesis is in turn controlled by a higher order regulatory mechanism.

The efficient induction of nuclear-mt stringency by cycloheximide, and by temperature shift in ts mutants defective in cytoplasmic protein synthesis, suggest that the mediating factor(s) in each genome may be proteins (e.g., 9, 10). This is consistent with our unpublished observation of a full and coordinate stringent response in a tight ts mutant for RNA polymerase II (33), when it is raised to the nonpermissive temperature. The protein responsible for governing mt transcription during a stringent response could be the specificity factor of the mt RNA polymerase (32, 34), or it may be some as yet unidentified protein. We are now attempting to identify and characterize the factor or factors which regulate stringent rDNA transcription in mitochondria.

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