Physiological alteration of the pattern of transcription of the *oli2* region of yeast mitochondrial DNA

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Transcription pattern shift, in mit⁻ oli2 *region* *ATP level, role in mitochondrial transcription*
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1. INTRODUCTION

Recent studies on the transcription of mitochondrial DNA (mtDNA) in *Saccharomyces cerevisiae* have revealed the pattern of a family of transcripts associated with each of the genes [1,2]. In studying the relationships between various members of a family of transcripts two approaches have proved useful. These are based on either the genetic or physiological perturbation of the transcription of a given region of mtDNA. The analysis of the processing events in the production of the mRNA from the mosaic *cyb* (*COB–BOX*) gene has been greatly assisted by the use of *mit*⁻ mutants in which the processing of RNA is blocked at different steps [3,4]. The accumulation and processing of 21 S rRNA precursors [5] have been studied in isolated mitochondria [6]. Modulation of the ATP concentration in the incubation medium during transcription showed that adenine nucleotides play an important role in the 21 S rRNA processing pathway.

The *oli2* gene codes for subunit 6 of the mitochondrial ATPase complex [7,8]. Detailed analysis of the transcription of the *oli2* region of yeast mtDNA [9] revealed at least 9 discrete transcripts ranging in size from 5.1–0.6 kilobases (kb). The 4 largest transcripts include the coding region of the *oli2* gene whilst the 4 smallest RNA molecules are derived from sequences downstream of the *oli2* gene [9]. A 4.5 kb species is thought to be the *oli2* mRNA since it is the most abundant transcript observed in Northern blots of mitochondrial RNA (mtRNA) probed with labelled mtDNA containing *oli2* gene sequences [9,10]. To try to determine the relationship of these *oli2* region transcripts to one another, we have studied the pattern of transcription of the *oli2* region in a number of *mit*⁻ strains carrying mutations either in the *oli2* gene or elsewhere on the mtDNA genome. The results presented here show that in all the *mit*⁻ mutants studied here there is a shift in the pattern of transcripts derived from the *oli2* region, such that the smallest transcripts are greatly reduced in abundance in favour of the largest 2 transcripts (5.1 and 4.5 kb in size). This pattern can be mimicked by treating wild-type cells with drugs that block mitochondrial development or function. We propose that this perturbation in vivo of transcription of the *oli2* gene results from a depletion in the ATP concentration in mitochondria of the *mit*⁻ mutants or of the antibiotic treated wild-type cells.

2. MATERIALS AND METHODS

2.1. *Yeast strains*

The *mit*⁻ strains were derived from *Saccharomyces cerevisiae* J69-1B *α ade1 his [rho⁺]*. Mutants Mb12, Mal, M13-20, M18-5, M27-14 and Ma30 all carry mutations in the *oli2* gene [8]; M27 [11] and 37-16-6 [12] carry mutations in the *oxi3* and *cyb* genes, respectively. Petite mutants DS14 [13] and Z3.32 [14] have been described.

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2.2. *Growth of cells and mtDNA analysis*

The *mit*$^-$ cells were grown in glucose-limited chemostat cultures [15]. Cells of strain J69-1B and the petite mutants were grown batchwise in medium [16] supplemented with auxotrophic requirements (each at 50 μg/ml) and containing glucose (2%) as carbon source. The preparation of mtRNA, analysis of mtRNA by electrophoresis, Northern transfer to diazotized paper and hybridization to ^{32}P-labelled DS14 mtDNA that was prepared by 3′-end labelling of *Eco*RI + *Hpa*II fragments of DS14 mtDNA have all been described [see 9].

3. RESULTS AND DISCUSSION

Fig.1 shows the pattern of transcripts of the *oli2* region in the wild-type strain J69-1B and the *mit*$^-$ mutants, as analyzed by Northern blots of mtRNA probed with labelled mtDNA from petite DS14 which covers the *oli2* gene and flanking regions. Whereas J69-1B (lane A) shows the 9 transcripts that we have described in [9], each of the *mit*$^-$ strains (lanes B–I) show only two clearly discernible bands of hybridization. These correspond in mobility to the 5100 and 4500 nucleotide species of J69-1B, but the relative intensities of hybridization are altered in the mutants. In J69-1B the 4500 nucleotide species hybridizes more intensely than the 5100 nucleotide species. By contrast, in many of the *mit*$^-$ strains the intensities appear about equal (lanes B,C,F–H) and some have the 5100 nucleotide species hybridizing more intensely than the 4500 nucleotide species (lanes D,E,I). Examination of the ethidium bromide-stained gel prior to transfer (not shown) showed that the mtRNA preparations from J69-1B and all *mit*$^-$ strains showed almost identical banding patterns. All preparations contained equivalent levels of the mitochondrial rRNAs and the contaminating cytoplasmic rRNAs. The mitochondria from the *mit*$^-$ strains were prepared from cells grown in glucose-limited chemostat cultures which minimises glucose repression and consequent fragility of mitochondria from respiratory-deficient cells [15].

The shift in the transcription pattern occurs in all the *mit*$^-$ strains regardless of the mutated locus (*oli2*, *oxi3* or *cyb*) and the phenotypic consequence of the mutation. Strains Mb12, Mal, M13-30, M18-3 and M27-14 all fail to synthesize subunit 6 of the mtATPase, and contain novel polypeptides of lower M_r which coprecipitate with antisera directed against the mtATPase complex [8]. The other *oli2 mit*$^-$ strain Ma30 lacks subunit 6 and contains no new polypeptides. Thus none of the above *oli2 mit*$^-$ strains has a specific defect in the transcription of the *oli2* region. It is inferred that the failure to translate the subunit 6 protein correctly in most of these *oli2* mutants results from nonsense mutations in the *oli2* coding region. Significantly, the shift in transcription pattern of the *oli2* gene (fig.1) also occurs in the *oxi3 mit*$^-$ mutant M27 and the *cyb mit*$^-$ mutant 37-16-6, both of which synthesize normal ATPase subunit 6. The presence of the 4500 and 5100 nucleotide species as the only detectable *oli2* transcripts in these strains strongly suggests that the mRNA of ATPase subunit 6 is one of these two species. This observation is consistent with our inference [9,10]

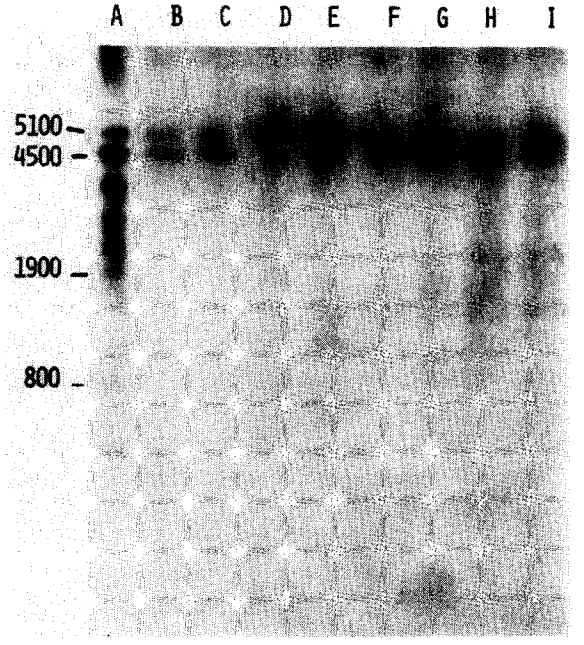

Fig.1. Analysis of *oli2* region transcripts in *mit*$^-$ strains. RNA from mitochondria was electrophoresed in a 1.5% agarose/6 M urea slab gel, transferred to diazotized paper and hybridized to ^{32}P-labelled mtDNA from petite DS14. Autoradiograms shown are for mtRNA from J69-1B (lane A), and *mit*$^-$ strains Mb12 (lane B), Mal (lane C), M13-20 (lane D), M18-5 (lane E), M27-14 (lane F), Ma30 (lane G), M27 (lane H), 37-16-6 (lane I). The positions of particular J69-1B *oli2* transcripts (measured sizes given in nucleotides) are indicated.

that the 4500 nucleotide species is the mRNA of ATPase subunit 6.

The *mit*⁻ mutants analyzed here show defects in the function of different mitochondrial enzyme complexes, namely ATPase (*oli2*), cytochrome oxidase (*oxi3*) or cytochrome *b* (*cyb*). It was thus considered that the equivalent perturbation of transcription of the *oli2* region in all cases resulted from a physiological change general to *mit*⁻ mutants. For example, a deficiency in the intramitochondrial level of ATP may be affecting the initiation or rate of transcription or the efficiency of RNA processing (cf. [6]). If this view is correct it would be predicted that the same perturbation of transcription of the *oli2* region would be observed in wild-type cells grown in the presence of drugs blocking mitochondrial development or function.

The results presented in fig.2 show that this is indeed the case. Cells cultured in the presence of the drugs antimycin A (lane C), carbonyl cyanide–*p*-trifluoromethoxyphenylhydrazone (CCCP) (lane D), erythromycin (lane E) and chloramphenicol (lane F) all show the same pattern of transcription as in the *mit*⁻ strains (cf. lane B). Antimycin A blocks mitochondrial respiration, while CCCP uncouples oxidative phosphorylation. These drugs mimic *mit*⁻ mutations at the *cyb* and *oli2* loci, respectively. The protein synthesis inhibitors erythromycin and chloramphenicol prevent the assembly of the respiratory chain itself. In all cases, intramitochondrial ATP synthesis is minimal. Previous studies with petite mutants [2] have indicated that precursors to mature species of yeast mtRNA are more readily detected in petites than in respiratory competent cells. These observations may now be rationalized in terms of the arguments presented above in that the diminished intramitochondrial ATP levels slow down the production of mature transcripts. This is illustrated here for the *oli2* gene by use of petite Z3.32, whose mtDNA genome covers a region including the *oli2* gene, extending at least 3 kb upstream and downstream of *oli2* [17]. The *oli2* transcripts in this petite (fig.2, lane G) do show the 5100 nucleotide transcript to be more prominent than the 4500 nucleotide transcript. In addition, the next 3 smaller *oli2* transcripts [9] (3900, 3600 and 3000 nucleotides) can also be observed. The observation of some smaller transcripts is considered to result from the amplification of the segment of the mtDNA genome retained in Z3.32 cells, which leads to an elevated level of transcription of this region relative to the wild-type and *mit*⁻ cells (cf. fig.2, lanes A and B).

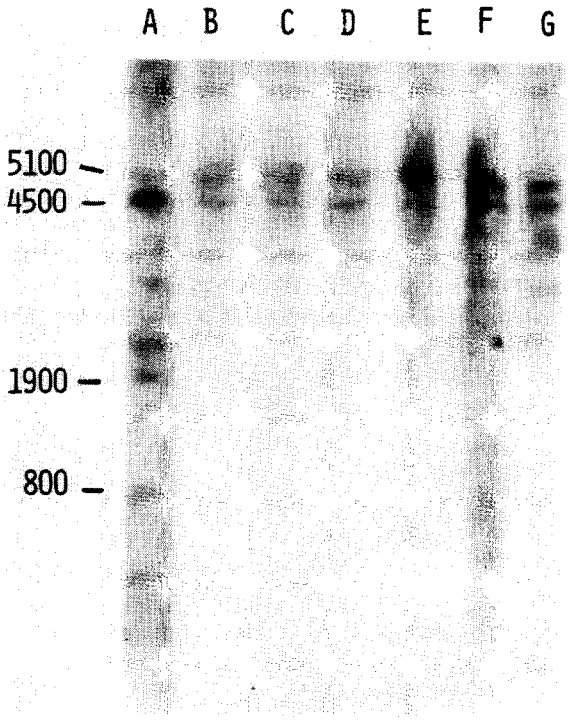

Fig.2. Analysis of *oli2* region transcripts in drug-treated cells. RNA from mitochondria was analysed as in fig.1. Autoradiograms shown are for mtRNA from J69-1B (lane A); Ma30 (lane B); J69-1B grown in the presence of the following drugs: antimycin A (0.1 mg/ml) (lane C); CCCP (2.4 mg/ml) (lane D), erythromycin (1 mg/ml) (lane E), chloramphenicol (4 mg/ml) (lane F), petite mutant Z3.32 (lane G). Other indications as for fig.1.

These results are consistent with the view that the smaller *oli2* transcripts arise from the larger transcripts by processing events. Alternative schemes involving multiple initiation sites for transcription [9] would fit these data if the efficiency of promotion at different sites is critically dependent upon the physiological state of the mitochondria. Although the ATP concentration is suggested to be the critical parameter in the in vivo perturbations described herein, the effects on transcription may themselves derive from secondary effects of lowered ATP levels, such as the structural organization of the mitochondrial transcription complex [18]. Nonetheless, the results presented here emphasize the importance of the state of development

of mitochondria [18,19] in controlling the transcription of yeast mtDNA in vivo.

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