# PREFERENTIAL TRANSCRIPTION OF dG + dC RICH MITOCHONDRIAL DNA IN CYTOPLASMIC PETITE MUTANTS OF SACCHAROMYCES CEREVISIAE

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Summary: Cytoplasmic $\rho^-$ petite strains of Saccharomyces cerevisiae were chemostat cultured to induce maximal synthesis of mtRNA. DNA buoyant densities and RNA base compositions were determined. The G + C content and U/A ratio of the RNA increased as the dG + dC content of the DNA decreased. The results obtained suggest the preferential transcription of dG +dC rich mtDNA in $\rho^-$ petites.

The respiratory deficient cytoplasmic petite mutants of Saccharomyces cerevisiae are of two basic types, those lacking in mtDNA (neutral $\rho^0$ petites) (1,2) and those containing mtDNA albeit of often grossly abnormal base composition (suppressive $\rho^-$ petites) (3,4). In the case of $\rho^0$ petites the loss of mtDNA results in the unitary loss of all mitochondrially coded products including the RNA translation components of the mitochondrial protein synthesizing system. In contrast with $\rho^0$ petites the $\rho^-$ mutation is nonunitary, as evidenced by the variable retention of gene markers such as erythromycin resistance (5) and differences in mtDNA base composition (3,4). In further contrast $\rho^-$ mutants contain mtRNA (6,7), although the nature of such RNA is largely unknown.

The present communication reports studies of the base composition of mtRNA in a number of $\rho^-$ mutants differing in ploidy, suppressiveness and retention of the erythromycin resistance determinant. There was an unexpected increase in the G + C content and U/A ratio of the RNA despite

a decrease in the dG + dC content of the mtDNA. The data suggests therefore that $\rho^-$ mtRNA polymerase is restricted to regions of higher dG + dC content as bulk dG + dC content falls.

## STRAINS

The various strains employed were kindly donated by Dr. A.W. Linnane, Monash University, Australia. $M\rho^+$ is a diploid prototrophic strain. $M\rho^-$ is a petite strain derived by treating $M\rho^+$ with euflavine. $L411\rho^+$ (α ur his ery 1-r) is a haploid erythromycin resistant strain (8). K1, K4, K5-1 and K5-2 are spontaneous $L411\ \rho^-$ strains. E5 is an $L411\rho^o$ ethidium bromide derived strain lacking mtDNA (1).

## RESULTS

Table I shows the buoyant density of the various mtDNA species together with the suppressiveness and degree of retention of erythromycin resistance in the $\rho^-$ petites isolated from strain L411. The buoyant density of the mtDNA in both the $M\ \rho^+$ and $L411\rho^+$ strains was 1.683 $g.cm^{-3}$. A decrease in buoyant density of 9 mg to 1.674 $g.cm^{-3}$ was found with the euflavine derived $M\ \rho^-$ strain, and 6 mg to 1.677 $g.cm^{-3}$ in all instances with the spontaneously derived $L411\rho^-$ strains. In the limited number of petites investigated there was no correlation between suppressiveness and erythromycin resistance, or between DNA buoyant density and either suppressiveness or retention of erythromycin resistance.

Table II shows the mtRNA content of the various strains. RNA levels in derepressed $\rho^-$ mitochondria were in all instances higher than in $\rho^+$ mitochondria, a difference due not to residual repression of $\rho^+$ cells but to the accentuated derepression observed when petite strains are chemostat

TABLE I

BUOYANT DENSITY OF MITOCHONDRIAL DNA IN WILD-TYPE AND PETITE CELLS, AND THE SUPPRESSIVENESS AND RETENTION OF ERYTHROMYCIN RESISTANCE IN L411$\rho^-$ PETITES

| Strain | Erythromycin Resistance (percent) | Degree of Suppressiveness (percent) | DNA Buoyant Density ($gm/cm^3$) |
|---|---|---|---|
| M$\rho^+$ | - | - | 1.683 |
| M$\rho^-$ | - | - | 1.674 |
| L411$\rho^+$ | 100 | - | 1.683 |
| L411$\rho^-$ | | | |
| K1 | 0 | 2 | 1.677 |
| K5-1 | 50 | 45 | 1.677 |
| K5-2 | 2 | 60 | 1.677 |
| K4 | 0 | 80 | 1.677 |

Wild-type grande M$\rho^+$ and L411$\rho^+$ cells were batch grown in basic 1% glucose - 1% yeast extract - 0.1% peptone medium (9). Petite strains were chemostat grown in the same basic medium for a minimum of 44 hours at dilution rates ranging from 0.16 to 0.18 $hr^{-1}$ using 2% glucose as the limiting substrate (10). Erythromycin resistance and suppressiveness values were determined as earlier described (5). Protoplast derived mitochondria (9) were employed in DNA buoyant density (1) and RNA base composition (Table II) determinations.

cultured (10). The mtRNA content of $\rho^+$ strains grown in batch culture as described is not increased by lengthening the duration of the stationary phase, but rather is decreased, as is the case when cells are grown entirely on ethanol (11).

The G + C content of mtRNA in derepressed $\rho^-$ mitochondria was increased in relation to $\rho^+$ mtRNA, the rise being 13.9% in the M$\rho^-$ petite and from 8.0 to 12.6% in the L411$\rho^-$ petites. Significantly, the increase in G + C content in all the strains investigated was due to a disproportionate rise in guanosine content, the percent rise being from approximately two to six-fold

TABLE II.

MITOCHONDRIAL RNA BASE COMPOSITIONS IN WILD-TYPE AND PETITE CELLS DIFFERING IN mtDNA BUOYANT DENSITY, SUPPRESSIVENESS AND ERYTHROMYCIN RESISTANCE

| Strain | mtRNA Content (μg.mg protein) | Nucleotide(s) (mole per cent) C | A | G | U | G+C | Ratio U/A |
|---|---|---|---|---|---|---|---|
| $M\rho^+$ | 61 | 12.7 | 35.2 | 16.2 | 35.9 | 28.9 | 1.02 |
| $M\rho^-$ | 80 | 16.1 | 22.4 | 26.7 | 34.6 | 42.8 | 1.54 |
| $L411\rho^+$ | 44 | 15.2 | 31.9 | 19.9 | 33.2 | 35.1 | 1.04 |
| $L411\rho^-$ | | | | | | | |
| K1 | 83 | 16.9 | 24.4 | 27.6 | 31.3 | 44.5 | 1.28 |
| K5-1 | 63 | 16.4 | 24.8 | 26.7 | 31.6 | 43.1 | 1.27 |
| K5-2 | 58 | 18.7 | 24.1 | 27.3 | 34.4 | 46.0 | 1.24 |
| K4 | 87 | 18.8 | 23.4 | 28.9 | 28.9 | 47.7 | 1.23 |
| $L411\rho^0$ | | | | | | | |
| E5 | 7 | 17.4 | 25.5 | 28.6 | 28.5 | 46.0 | 1.12 |

Mitochondria were prepared as described in Table I. RNA nucleotide compositions were determined after precipitating the mitochondria and washing twice with 10% TCA, washing with ethanol, defatting for 15 min at 65°C in 1:1 ethanol-ether (V/V) and then drying in ether. Residual pellets were hydrolyzed in 0.3 M KOH for 16 hours at 37°C before the hydrolysates were neutralized with 12 M $HClO_4$, centrifuged, and chromatographed on Dowex-50 (ref. 12).

the percent rise in cytosine content. In similar vein the decrease in U +A content of the $\rho^-$ mtDNA was due to a disproportionate fall in the adenosine content (Table II).

The abnormal base composition and high G + C content of the various $\rho^-$ mtRNA hydrolyzates was not due to contamination of the mitochondrial preparations with cytoplasmic RNA. Firstly, the U/A ratios of the $\rho^-$ mitochondrial preparations (1.54 - 1.23) were always considerably higher than the U/A ratios of homologous cytoplasmic RNA, the mean values being 1.15

for M$\rho^+$/ $\rho^-$ cytoplasmic RNA, and 1.11 for L411$\rho^+$/$\rho^-$ cytoplasmic RNA. Secondly, RNA of unusual base composition was not found in control experiments employing the $\rho^o$ mtDNA-less E5 mutant. Low levels of RNA were invariably associated with E5 mitochondrial preparations no matter how carefully the mitochondria were washed, but the RNA base composition was always that of cytoplasmic RNA (Table II).

The derepression of cytochrome c synthesis in experiments involving the $\rho^o$ E5 mutant was of interest in that it demonstrates that mtDNA plays little if any role in the nucleo-cytoplasmic phenomenon of catabolite repression. The several fold increase in cytochrome c levels of chemostat grown $\rho^-$ cells has been noted previously (10).

## DISCUSSION

Mitochondria from batch grown $\rho^-$ petites have been reported to contain as little as 20% of the RNA found in $\rho^+$ cells (7). The data of this paper indicates however that such RNA levels are the result of catabolite repression rather than an intrinsic property of the $\rho^-$ mutation, since RNA levels increased manyfold when cells were chemostat cultured with glucose as the limiting substrate. Increased synthesis of mtRNA in derepressed $\rho^+$ strains has also been reported (13).

The nucleotide composition of the derepressed mtRNA indicates a marked restriction in the range of mtDNA transcribed in petite mitochondria. Furthermore, it appears that the greater the decrease in buoyant density the greater the increase in the G + C content and U/A ratio of the mtRNA. Several points are of interest. Thermal transition profiles and optical rotary dispersion studies have indicated that the dG + dC base pairs of $\rho^+$ mtDNA appear to

be clustered rather than distributed evenly along the DNA (14,15), and consideration of the nucleotide composition of the triplet codons has indicated that regions of at least 30% dG + dC would be essential if mtDNA were to code for proteins containing all 20 amino acids (16). In general agreement with these findings, the G + C content of even highly purified $\rho^+$ mtRNA is some 10% higher than the 17% dG + dC content of the bulk DNA. Even more strikingly, the G + C content of whole organelle $\rho^+$ mtRNA is frequently in the range 30-35% even after mitochondria have been treated with RN'ase and gradient purified in order to remove contaminating cytoplasmic RNA. The abnormally high G +C content of the $\rho^-$ mtRNA described in this communication may accordingly be taken as further evidence for the existence of dG + dC clusters in mtDNA. Furthermore, the base content of the $\rho^-$ mtRNA suggests that the dG + dC composition of at least some of these clusters must be in excess of 40%.

The process by which the G + C content and U/A ratio of the $\rho^-$ mtRNA increases as the mtDNA buoyant density decreases is not clear. The retention of erythromycin resistance in some of the mutants indicates however that at least part of the DNA is functional. In similar vein the retention of the $tRNA_{ser}$ codon in a mutant containing mtDNA of 3.6% dG + dC content has been reported (17), as has the apparent normality of up to 50% of the mtDNA of other $\rho^-$ strains (18). Subsequent fragmentation of the DNA notwithstanding (2,19) it would therefore seem that decreases in DNA buoyant density represent deletion of dG + dC base pairs from localized regions of DNA rather than evenly distributed deletions extending over the entire length of the molecule.

The increase in G + C content of the $\rho^-$ mtRNA hints then that the initial loss of dG - dC base pairs commences in a region of lower than average dG +dC content, and that transcription is confined to regions of progressively

higher dG + dC content as buoyant density decreases. The maximum possible increase in the G + C content of $\rho^-$ RNA in petites of progressively lower DNA buoyant density would therefore be of interest in that regions of DNA least susceptible to damage might well be poly dG - poly dC sequences of the type implicated in the control of transcription in microbial systems. Indeed, it is tempting to speculate that the high GMP content of $\rho^-$ mtRNA represents at least in part the incomplete products of the repetitive transcription of short but essentially normal regions of heavy-strand DNA associated with poly-dC initiation regions. The abnormally low AMP content of the RNA is consistent with this suggestion, and may furthermore indicate a relative inability of mtRNA polymerase to transcribe sections of DNA of abnormally high dT content.

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