Overexpression of the yeast transcriptional activator ADR1 induces mutation of the mitochondrial genome

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Summary. It was previously observed that increased dosages of the \textit{ADR1} gene, which encodes a yeast transcriptional activator required for alcohol dehydrogenase II (ADH II) expression, cause a decreased rate of growth in medium containing ethanol as the carbon source. Here we show that the observed reduction in growth rate is mediated by the ADR1 protein which, when overexpressed, increases the frequency of cytoplasmic petites. Unlike previously characterized mutations known to potentiate petite formation, the ADR1 effect is dominant, with the petite frequency rising concomitantly with increasing ADR1 dosage. The ability of ADR1 to increase the frequency of mitochondrial mutation is correlated with its ability to activate \textit{ADHII} transcription but is independent of the level of \textit{ADHII} being expressed. Based on restoration tests using characterized \textit{mit} strains, ADR1 appears to cause non-specific deletions within the mitochondrial genome to produce \textit{rho}- petites. Pedigree analysis of ADR1-overproducing strains indicates that only daughter cells become petite. This pattern is analogous to that observed for petite induction by growth at elevated temperature and by treatment with the acridine dye euflavine. One strain resistant to ADR1-induced petite formation displayed cross-resistance to petite mutation by growth at elevated temperature and euflavine treatment, yet was susceptible to petite induction by ethidium bromide. These results suggest that ADR1 overexpression disrupts the fidelity of mitochondrial DNA replication or repair.

Key words: Mitochondrial mutation – Transcriptional activation – ADR1

Introduction

\textit{Saccharomyces cerevisiae} has long been known to be highly susceptible to mitochondrial mutations which lead to the formation of respiratory deficient "petite" cells (Euphrussi 1953). Petites lack (\textit{rho}°) or have significant deletions (\textit{rho}−) in their mitochondrial DNA (mtDNA) and are unable to grow on nonfermentable carbon sources due to a loss of mitochondrial function. Petites also grow more slowly on fermentable carbon sources than \textit{rho}+ or "grande" cells. Yeast strains normally produce petites spontaneously at a rate of about 1–2% per cell generation. This rate is increased if cells are grown at elevated temperatures (≥37°C, Sherman 1959) or in the presence of mutagenic dyes such as euflavine or ethidium bromide (reviewed by Gillham 1978).

In addition to growth conditions, numerous recessive nuclear mutations have also been shown to increase the rate of spontaneous mtDNA mutations. The \textit{pet} mutations directly disrupt nuclearily encoded mitochondrial structures necessary for mitochondrial function (e.g., membrane or ribosomal components) and, for reasons unknown, cause the mitochondrial genome to mutate more frequently (Sherman and Slonimski 1964). Others, such as the \textit{gam1}, 2, 3 and 4 (Foury and Goffeau 1979), \textit{mmc} (Marmiroli et al. 1980), and \textit{cdc8} and 21 (Newlon et al. 1979) mutations, cause disruptions in the replication, recombination, and repair processes required for the maintenance of mtDNA (reviewed by Moustacchi and Heude 1981) and thus more directly alter the stability of the mitochondrial genome.

The ADR1 protein is the primary transcriptional activator of the glucose-repressible alcohol dehydrogenase (ADH II: encoded by the \textit{ADH2} gene) in \textit{S. cerevisiae}. Under glucose growth conditions \textit{ADH2} is not transcribed. When glucose is depleted there is a dramatic \textit{ADR1}-dependent rise in the level of \textit{ADH2}
mRNA, leading to a several hundred-fold increase in ADH II enzyme activity (Denis et al. 1981). Deletion studies on the ADH2 upstream region suggest that ADR1 facilitates transcription by binding to a region of dyad symmetry upstream of ADH2 (Shuster et al. 1986).

ADR1 has been cloned and sequenced (Denis and Young 1983; Hartshorne et al. 1986) and is predicted to encode a polypeptide of 1,323 amino acids. The regions thought to be important to ADR1 function include two putative DNA-binding zinc fingers (Miller et al. 1985) and a cAMP-dependent protein kinase phosphorylation consensus sequence (Kemp et al. 1977). The importance of the putative phosphorylation sequence is evidenced by the constitutive mutation ADR1-5°. ADR1-5° encodes a single amino acid substitution within the phosphorylation consensus sequence which causes a decrease in the level of ADR1 phosphorylation (Denis and Gallo 1986; Cherry et al. 1989). Yeast strains carrying the ADR1-5° allele partially escape glucose repression, presumably due to a lower level of phosphorylation of ADR1-5° relative to that of the wild-type protein.

While ADR1 does not affect the transcription of several other glucose-regulated genes (unpublished data), it is required for growth in medium containing glycerol as the carbon source (Bemis and Denis 1988). The effect of ADR1 on glycerol growth is, however, not general for non-fermentative processes, since the ADR1 gene is not required for growth on ethanol-containing medium. It was therefore surprising to discover that overexpression of the nuclear regulatory protein ADR1, having no known functions involving the mitochondria, induces mutation of the mitochondrial genome.

Materials and methods

Media and yeast strains. YEP medium contains 2% bactopeptone, 1% yeast extract, 20 mg/l adenine and uracil supplemented with 8% glucose (YEP-gl) or 3% ethanol (YEP-Et). YEP plates contain YEP medium supplemented with 2% bactoagar and 2% glucose or 3% ethanol. Ethidium bromide and acriflavin were purchased from Sigma (St. Louis, Mo) and were added to liquid medium from freshly prepared stock solutions (1 mg/ml). Acriflavin is a commercial mix of proflavine (2,8-diaminoacridine) and ethidium (10-methyl-2,8-diaminoacridine) in a ratio of ca. 1:2 (proflavine is relatively inert compared to ethidium (Mattick and Nagley 1977)). Derivatives of strain 500-16 (MATa adh1-11 adh3 adh1-1 trpl ura1 his4) carrying multiple copies of the ADR1 or ADR1-5° alleles integrated into the genome have been described (Denis 1987). These strains contain complete ADR1 or ADR1-5° sequences in plasmid YRp7 which have been tandemly integrated at the trp1 or adh1-1 locus. They are designated 411-40, -20, and -6 (1, 20, and 75 copies of ADR1) or 60-60, -6, and -2 (1, 5, and 8 copies of ADR1-5°). Other strains used in this study include 604-6a (MATa adh2 adh3 trpl ur2 his4) and 233-2b (MATa adh1-11 ADH2-4 adh3 trpl ura1 leu2).

Determination of petite frequencies. Petite frequencies were determined by two methods which gave comparable values: liquid culture dilution and plate colony dilution. In the liquid culture dilution procedure single colonies were picked and grown to mid-log phase in YEP-gl medium. Cultures were diluted and plated on YEP-gl plates to give between 100 and 400 colonies per plate. Petites were distinguished from grands after three days growth by their smaller size and whiter color. The plate colony dilution method consisted of suspending single colonies from YEP-gl plates in sterile water, making an appropriate dilution, and replating on YEP-gl plates. Colonies thought to be petite based on size and color were routinely checked for growth on non-fermentable carbon sources and assayed for ADH II activity to ensure that the ADR1 gene dosage had been maintained. Frequencies are expressed as the percentage of total cells plated (100-400) which were petite and are the average of at least three independent determinations. The average variation of independent determinations of petite frequencies was 20% except for petite frequencies that were less than 5%.

Pedigree analysis. Cells to be analyzed were pregrown overnight in YEP-Et medium at 30°C to ensure that only grande cells served as mothers. Cells were transferred to YEP-gl plates where buds were dissected from mother cells by micromanipulation. Single daughter cells were placed at designated positions on the agar and colonies arising from these cells were scored for the petite phenotype by growth on YEP-Et plates.

Restoration tests using characterized mit- strains. mit- strains are mutants which can carry out mitochondrial protein synthesis but have defined defects in mitochondrial genes. ADR1-induced petites were crossed to three tester strains containing mutations or small deletions in the OX13, COB or OX12 mitochondrial genes. The mit- tester strains M10-50, M7-40 and M9-3 have been described elsewhere (Slonimski and Tzagoloff 1976). Respiraton-deficient petites containing mtDNA capable of recombining with a mit- tester to form a functional mitochondrial genome would be expected to result in diploids possessing restored respiratory function. Diploids were scored for restoration by growth on YEP-Et plates.

Results

Overexpression of ADR1 induces petite formation

It was previously observed that strains containing high dosages of ADR1 integrated into the genome grow slowly in medium containing ethanol as the carbon source (Denis 1987). This result was surprising since high ADR1 copy number is known to increase ADH II activity (Denis 1987), which would be expected to facilitate rather than hinder growth in ethanol-containing medium. Strains carrying high dosages of ADR1, when plated to single colonies, yielded a heterogenous population of cells comprised of large, sectored, amber colonies and small, round, white colonies (Fig. 1). The small white colonies were determined to be cytoplasmic petites by three criteria: 1) inability to grow on non-fermentable carbon sources, 2) lack of staining by the TTC overlay technique of Ogur et al. (1957), and 3) non-Mendelian segregation of the petite phenotype (data not shown).