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EXPRESSION OF MITOCHONDRIAL DNA IN SACCHAROMYCES CEREVISIAE: THE CONSTRUCTION OF SETS OF ISONUCLEAR HAPLOID STRAINS CONTAINING DIFFERENT SPECIFIED MITOCHONDRIAL GENOMES

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SUMMARY: In order to perform systematic biochemical studies of the expression of the mitochondrial genome in different strains of Saccharomyces cerevisiae, we have constructed a series of haploid strains in which a specified mitochondrial genome has been placed in cells containing a particular nuclear genome. The desired strains were obtained from crosses between rho^+ and rho° haploids, one of which carried the kar1-1 mutation which interferes with nuclear fusion. These newly constructed strains provide the basis for studying mitochondrial gene expression as a function of (i) the nucleotide sequence differences that are apparent in the mitochondrial DNAs from different strains, and (ii) the role played by different nuclear genomes in modulating the expression of mitochondrial genes.

INTRODUCTION

The mitochondrial genome of yeast (Saccharomyces cerevisiae) is the subject of intensive investigation at many levels (see e.g. 1, 2). From both genetic (3) and physical studies (4) on mtDNA from different strains of yeast it became apparent that there are significant differences in the mitochondrial genomes of different strains (see also ref. 1). Detailed studies on mtDNA using restriction endonucleases showed that these sequence differences arise from insertions and deletions at many places on the mitochondrial genome (5, 6). These observations raise the general question as to the consequences that these widespread sequence differences would have on the expression of the genetic information encoded in mtDNA.

In order to carry out a systematic study of the differences in mitochondrial gene expression resulting from this variation in mtDNA sequences, an important condition should be met. This condition is that the diverse mitochondrial genomes must all be studied in a common nuclear background, in order to overcome possible strong nuclear influences known to affect

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the replication and expression of yeast mtDNA (1). It thus becomes necessary to construct a set of isonuclear strains containing different mitochondrial genomes. This is not possible using conventional methods of genetic analysis in S. cerevisiae. However, the recent discovery made by Conde and Fink (7) of a nuclear mutation (kar1-1) which leads to defects in the fusion of the haploid nuclei during zygote formation has extended the range of genetic manipulation of yeast. Haploid cells containing the kar1-1 mutation can mate with haploids of opposite mating type to produce heterokaryons, which subsequently segregate haploid cells containing one of the parental haploid nuclei together with the mixed cytoplasm from both parents. We reasoned that if one of the parents is a petite of the rho° type, lacking mtDNA (8, 9), and the other parent is a respiratory competent (grande or rho$^+$) strain, then it becomes possible to transfer the mtDNA of the rho$^+$ strain to haploid cells containing the nucleus of the rho° strain.

In this communication we describe the construction of haploid strains in which a specified mitochondrial genome is placed in cells containing a particular nuclear genome. We present the results of experiments which demonstrate, at a genetic level, the value of these newly constructed strains in studying the expression of different mtDNAs, and the role played by different nuclear genomes in modulating mitochondrial gene expression.

MATERIALS AND METHODS

Strains: The grande (rho$^+$) strains, and petite strains lacking mtDNA (rho°), used in this work are listed in Table 1.

Media: YEPD plates were made with Difco yeast extract (1%), Bacto-peptone (2%), glucose (2%), agar (1.5%). YEPE plates contained yeast extract (1%), peptone (2%), ethanol (2%), agar (1.5%). SD plates contained Difco yeast nitrogen base (without amino acids) (0.67%), glucose (2%), agar (1.5%). Where appropriate, the SD plates were supplemented with various amino acids, adenine or uracil (10).

Crosses involving kar1-1 mutants: In each case a rho$^+$ haploid was crossed to a rho° haploid; one of the haploids carried the mutant kar1 allele (7). The objective of this procedure was to isolate from the progeny of the cross a rho$^+$ haploid carrying the nuclear genetic markers of the rho° parent (cf. 7, 11). A sample of each of the parent strains was grown

TABLE 1. LIST OF STRAINS

Strain	Nuclear genotype	Mitochondrial genotype	Source
1. Grande strains			
JC6	α his4-15 ade2-1 leu1 kar1-1	rho$^+$	Ref. 7
770-7B	a arg4-17 lys2 trp1	rho$^+$ cap1-r ery1-r oli1-r ana1-r	Ref. 12
761-7A	a arg4-17 lys2 ura	rho$^+$ cap1-r ery1-r oli1-r	Ref. 12
432-31	a ura trp1	rho$^+$ ery1-r oli1-r ana1-r	Ref. 12
2. Petite Strains[a]			
BT1-9	α his4-15 ade2-1 leu1 kar1-1	rho°	JC6
EU-0	a arg4-17 lys2 trp1	rho°	770-7B
EP-0	a arg4-17 lys2 ura	rho°	761-7A
ES-0	a ura trp1	rho°	432-31

a: Petite strains listed here are all of the rho° type lacking mtDNA (9) and were derived from the rho$^+$ parent strains by ethidium bromide treatment (8).

overnight on a YEPD plate. A portion of each parent haploid culture was mixed on the surface of a YEPD plate. The mating mixture was kept at 28° for 6-8 h, after which a portion was transferred to a YEPE plate, and allowed to grow at 28° for 2-3 days, in order to enrich the cell population for rho$^+$ cells. A sample of this YEPE culture was suspended in sterile water, and after dilution in water, portions were spread on SD plates supplemented with the auxotrophic requirements of the rho° parent strain. After 3-5 days at 28°, both small and large colonies were found. The colonies on a supplemented SD plate were replica plated by the velveteen method onto each of the following plates: SD, YEPE and YEPD. The desired rho$^+$ haploids were selected as those colonies which do not grow on unsupplemented SD medium, but which show growth on YEPE plates. These colonies were found amongst the smaller colonies on the supplemented SD plate. The other major classes of colonies on the supplemented SD plates which it was necessary to screen out were (i) stable diploid clones (generally large colonies) which showed strong growth on SD medium, and (ii) petite haploids, which were small colonies unable to grow on either YEPE or unsupplemented SD plates. There were apparently few unstable heterokaryons (7) left in the progeny of the cross after the growth period on YEPE. The frequency of rho$^+$ haploids was usually between 2 and 50% of colonies recovered on the supplemented SD plates. Putative rho$^+$ haploids were selected from each cross and tested for nuclear markers and mitochondrial drug resistance genes (see Table 2 for procedures).

TABLE 2. GRANDE HAPLOID STRAINS OBTAINED BY CROSSING *kar1-1* AND *KAR1*$^+$ HAPLOIDS [a]

Strain	Parent strains *rho*°	Parent strains *rho*$^+$	Auxotrophic requirements [b]	Antibiotic resistance [c]
PK2-5	BT1-9	770-7B	his$^-$ade$^-$leu$^-$	cap-r ery-r oli-r ana-r
PK4-2	BT1-9	761-7A	his$^-$ade$^-$leu$^-$	cap-r ery-r oli-r
PK8-1	BT1-9	432-3l	his$^-$ade$^-$leu$^-$	ery-r oli-r ana-r
PK9-5	EU-0	JC6	arg$^-$lys$^-$trp$^-$	all-s
PK10-2	EP-0	JC6	arg$^-$lys$^-$ura$^-$	all-s
PK11-3	ES-0	JC6	ura$^-$trp$^-$	all-s

a: Strains BT1-9 and JC6 carry the mutant allele *kar1-1* (Table 1); other strains are of genotype *KAR1*$^+$ (wild-type at this locus).

b: Auxotrophic requirements were checked by dropping suspensions of the strains onto SD plates containing appropriate supplements (10).

c: Antibiotic resistance was tested by dropping suspensions of the strains onto YEPE plates containing one of the following antibiotics: chloramphenicol (2 mg/ml), erythromycin (1 mg/ml), oligomycin (3 µg/ml), antimycin A (0.01 µg/ml); all-s indicates the strain was sensitive to all drugs tested.

RESULTS AND DISCUSSION

The general procedure described here for constructing haploid strains carrying the nucleus of one strain and the mitochondrial genome of another strain has been used successfully with many strains in our laboratory. Some examples are shown in Table 2. It can be seen in every case that the selected *rho*$^+$ haploid colonies contained the nuclear auxotrophic requirements of the *rho*° haploid parent, and the mitochondrial drug resistances characteristic of the *rho*$^+$ haploid parent (cf. Table 1). The use of a *rho*° haploid (lacking mtDNA) in these crosses ensured that the *rho*$^+$ progeny contained only the mtDNA of the *rho*$^+$ parent. It should be noted that it does not matter whether the *rho*° or the *rho*$^+$ haploid parent carries the *kar1* mutation; the crosses can be carried out in either configuration (see Table 2).

Considering the constructed strains noted in Table 2 it is seen that the first three strains represent a set of isonuclear haploids each containing a different mitochondrial genome, all with the nucleus of JC6. Any phenotypic differences between these strains and between strain JC6 itself must be due to differences in the mtDNA. A set of strains of this type provides the basis for a comparative study of mitochondrial gene expression. Moreover any nuclear influences on mitochondrial gene expression can be studied by comparing each of these newly constructed haploids (containing the JC6 nucleus), with the respective rho$^+$ parent strain (e.g. PK2-5 and 770-7B). Furthermore, there is opportunity to transfer any of these mitochondrial genomes to cells containing almost any other haploid nucleus, by crossing the rho$^+$ haploids with the JC6 nucleus (kar1-1) to another rho$^\circ$ strain. If desired the whole set of mitochondrial genomes can be transferred to a new reference nuclear genetic background.

It is instructive to consider the results of some of these genetic manipulations in terms of a simple phenotypic test for mitochondrial gene expression; namely, the growth of yeast cells on YEPE medium, containing the non-fermentable substrate ethanol. Molloy et al. (3) previously reported that strains 770-7B and 761-7A showed a reduced rate of growth on YEPE at 28°, and some evidence was obtained for the mitochondrial inheritance of this slow growth character (3). We extended these studies by considering the growth of strains JC6, 770-7B, 432-31, 761-7A, and derived strains, on YEPE medium at 18°, 28° and 36°, as shown in Table 3. Analysis of the growth of the four rho$^+$ parent strains shows that whilst JC6 grows strongly on YEPE at all temperatures tested, strain 770-7B grows slowly on YEPE at 28° and is even more markedly inhibited at 18° and 36°. Strain 432-31 shows a cold-sensitivity manifested in a reduced growth at 18° (Table 3) but normal behaviour at 28° and 36°. Finally, strain 761-7A resembles 770-7B in showing markedly reduced growth at all temperatures

TABLE 3. GROWTH OF STRAINS AT DIFFERENT TEMPERATURES WITH ETHANOL AS CARBON SOURCE

Strain	Source of nucleus	Source of mitochondrion	Growth [a] 18°	28°	36°
JC6	JC6	JC6	4+	4+	4+
770-7B	770-7B	770-7B	2+	3+	2+
432-31	432-31	432-31	2+	4+	4+
761-7A	761-7A	761-7A	1+	2+	2+
PK2-5	JC6	770-7B	0	2+	1+
PK9-5	770-7B	JC6	4+	4+	4+
PK8-1	JC6	432-31	1+	4+	3+
PK11-3	432-31	JC6	3+	4+	4+
PK4-2	JC6	761-7A	1+	2+	1+
PK10-2	761-7A	JC6	0	2+	1+

a: Growth was determined by dropping cell suspensions onto YEPE plates, and incubating the plates for 5 days at the indicated temperature. The extent of growth of each patch is expressed on an arbitrary scale (from 0 to 4+), with the maximal growth at each temperature of a non-temperature sensitive strain (e.g. JC6) defined as 4+. These strains showed no temperature sensitivity for growth on YEPD.

tested. Analysis of strains constructed using karl-1 mutants provides insight into the role played by the mitochondrial and nuclear genomes in the slow growth and temperature sensitive properties of these parent strains.

Strain PK2-5 was constructed so as to contain the nucleus of JC6 and the mtDNA of 770-7B; strain PK2-5 is highly cold and heat-sensitive (showing little or no growth at 18° and 36° on YEPE) and grows very slowly at 28° (Table 3). However, strain PK9-5 (770-7B nucleus, JC6 mtDNA) grows normally at all temperatures. The defect in 770-7B is clearly attributable entirely to its mitochondrial genome. Furthermore, the JC6 nucleus is seen to modify the expression of the 770-7B mtDNA so as to exacerbate the slow growth and temperature sensitivity (cf. strains PK2-5 and 770-7B in Table 3).

Considering the cold-sensitivity of strain 432-31, it can be concluded that this property is a function of its mtDNA; the constructed strain PK8-1 (JC6 nucleus, 432-31 mtDNA) is markedly cold-sensitive (Table 3). The 432-31 mtDNA also specifies a mild heat-sensitivity (not seen in strain 432-31 itself) as evidenced by the slightly reduced growth of strain PK8-1 on YEPE at 36°. The properties of the constructed strain PK11-3 (432-31 nucleus, JC6 mtDNA) show that the 432-31 nucleus may play a minor role in determining the cold-sensitivity of strain 432-31, as evidenced by the slight cold-sensitivity of strain PK11-3 (Table 3).

The genetic specification of the slow growth of strain 761-7A was found to be complex. Both the constructed haploids PK4-2 (JC6 nucleus, 761-7A mtDNA) and PK10-2 (761-7A nucleus, JC6 mtDNA) show slow growth at 28° and marked cold and heat-sensitivity. Thus there is a strong contribution from both the nuclear DNA and mtDNA of 761-7A to the reduced growth on YEPE.

The investigation of the molecular basis of these slow growth effects will form part of a wider study of sets of isonuclear strains that are now being used for a systematic analysis of mitochondrial gene expression resulting from sequence variation in grande mtDNA. The isonuclear set based on JC6 (cf. Table 2) is currently being extended in our laboratory and we now have a set of fourteen strains all with the JC6 nucleus and the mitochondrial genomes from a wide variety of other yeast strains, selected for differences in the restriction enzyme digestion patterns of their mtDNA. The results of these studies on mitochondrial gene expression will be reported elsewhere.

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REFERENCES

1. Nagley, P., Sriprakash, K.S., and Linnane, A.W. (1977) in Advances in Microbial Physiology (Rose, A.H., and Tempest, D.W., eds.), vol. 16, pp.157-277, Academic Press, London.

2. Bandlow, W., Schweyen, R.J., Wolf, K., and Kaudewitz, F. (1977) eds. Mitochondria 1977: Genetics and Biogenesis of Mitochondria, de Gruyter, Berlin.
3. Molloy, P., Linnane, A.W., and Lukins, H.B. (1976) Genet. Res., Camb. 26, 319-325.
4. Bernardi, G., Prunell, A., and Kopecka, H. (1975) in Molecular Biology of Nucleocytoplasmic Relationships (Piuseux-Dao, S., ed.), pp.85-90, Elsevier, Amsterdam.
5. Sanders, J.P.M., Heyting, C., Di Franco, A., Borst, P., and Slonimski, P.P. (1976) in The Genetic Function of Mitochondrial DNA (Saccone, C., and Kroon, A.M., eds.), pp.259-271, North-Holland Publishing Co., Amsterdam.
6. Prunell, A., Kopecka, H., Strauss, F., and Bernardi, G. (1977) J. Mol. Biol. 110, 17-52.
7. Conde, J., and Fink, G.R. (1976) Proc. Nat. Acad. Sci. U.S.A. 73, 3651-3655.
8. Nagley, P., and Linnane, A.W. (1970) Biochem. Biophys. Res. Commun. 39, 989-996.
9. Nagley, P., and Linnane, A.W. (1972) J. Mol. Biol. 66, 181-193.
10. Linnane, A.W., Kellerman, G.M., and Lukins, H.B. (1975) in Techniques of Biochemical and Biophysical Morphology (Glick, D., and Rosenbaum, R., eds.), vol. 2, pp.1-98, John Wiley and Sons, New York.
11. Zakharov, I.A., and Yarovoy, B.P. (1977) Mol. Cell. Biochem. 14, 15-18.
12. Molloy, P.L., Linnane, A.W., and Lukins, H.B. (1975) J. Bact. 122, 7-18.