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Construction of isomitochondrial and isonuclear strains for recombinational analysis of mitochondrial loci in *S. cerevisiae*

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SUMMARY

In the mitochondrial and nuclear genetic background of the 777-3A strain (*op1*, *ade1*, α) a set of strains with different nutritional requirements and mating types, carrying the *op1* mutation versus its wild-type *OP1* allele was constructed. This makes possible the use of strains with the same genetic background in recombinational analysis of mitochondrial loci.

1. INTRODUCTION

It is well established that different yeast ρ^+ strains show many variations in their mitochondrial DNA sequence without the overall gene order being changed and without apparent ill effects on the synthesis of major gene products (for reference see Borst & Grivell, 1978). Thus, in fine structure analysis of mitochondrial loci it is desirable to deal with different mitochondrial mutants in the same nuclear and mitochondrial backgrounds. The subject of the present work was the construction of a set of isonuclear and isomitochondrial strains for recombinational analysis of *mit*$^-$ mutants belonging to *OXI1* and *OXI2* mitochondrial loci.

Probably the easiest way of isolating *mit*$^-$ mutants was that described by Kotylak & Slonimski (1977). They used strains carrying the *op1* mutation. The *op1* mutant, first described by Kováč Lachowicz & Slonimski (1967) is unable to grow on nonfermentable substrates. In the presence of the *op1* mutation, ρ^- mutations are lethal (Kováčová, Irmlerová & Kováč, 1968). Thus, all respiratory mitochondrial mutants isolated in the *op1* background are of *mit*$^-$ type. This method was used for the isolation of *mit*$^-$ mutants belonging to *OXI1* and *OXI2* loci.

For crossing the *mit*$^-$ mutants in all possible pairwise combinations, accompanied by selective screening of zygotic clones on minimal medium, it was necessary to obtain the mutants in strains of the opposite mating type with complementary nutritional requirements. Moreover, establishment of the frequency of *mit*$^+$ recombinants between two *mit*$^-$ mutants by their selective screening on complete medium with non-fermentable carbon source is possible only among the diploids with the *OP1* phenotype. Thus, at least one of the two haploid strains crossed should carry the wild-type *OP1* dominant allele of the *op1* mutation.

Mutants *oxi1* and *oxi2* were isolated in the *op1* strain 777-3A. It was, therefore, necessary to construct isonuclear and isomitochondrial derivatives of strain 777-3A, differing from that only in mating type, nutritional requirements and in carrying the wild-type allele of the *op1* mutation. This paper describes the construction of such strains.

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2. MATERIALS AND METHODS

(i) *Strains*. The list of original strains used is given in Table 1. Isonuclear and isomitochondrial derivatives of the 777-3A strain are shown in Fig. 1 and described in Basic Procedure and Results.

(ii) *Media*. The media used were the following. GO: minimal medium containing 2% glucose as a carbon source (see Galzy & Slonimski, 1957). GOade: GO supplemented with 20 mg/l adenine. VB: sporulation medium containing 0·82% CH_3COONa, 0·18% KCl, 0·17% NaCl, 0·035% $MgSO_4$. NO: glucose complete medium containing 1% Yeast Extract (Difco), 1% Becto Peptone (Difco), 2% glucose, 0·1 M Sörensen phosphate buffer pH 6·2. N3: glycerol complete medium, the same as NO but containing 2% glycerol instead of glucose. The last two media were prepared essentially according to Coen *et al.* (1970). All solid media were solidified with 2% Bacto-agar Difco.

Table 1. *List of strains*

Strain	Nuclear genotype	Mitochondrial genotype	Reference*
777-3A	α ade1	$\rho^+\omega^+$	1
IL8-8D	a ura1	$\rho^+\omega^+C^RE^R$	2
LK1-4C	a trp, op1	ρ^+	3
DT15/1	a his4A, leu2	ρ^0	4
SP4/1	α leu1, arg4	ρ^0	5

* 1, Kotylak and Slonimski (1977); 2, Dujon *et al.* (1976); 3, obtained from Dr Z. Kotylak; 4, BET induced ρ^0 mutant of DT15 (obtained from Dr G. R. Fink); 5, BET induced ρ^0 mutant of SP4 (Biliński *et al.* 1978).

(iii) *Tetrad analysis*. Sporulation was performed by incubation of diploid cells for 5–7 days at 30 °C on VB solid medium. Microdissection was performed as described by Mortimer & Hawthorne (1969).

(iv) *Mating procedure*. Crosses were performed using the standard cross technique described by Coen *et al.* (1970).

(v) *Abbreviations*. ρ^+/ρ^- – grande/cytoplasmic petite; ρ^0 – cytoplasmic petite mutant that is devoid of all mitochondrial DNA; ω^+ – one of the allelic forms of mitochondrial ω locus; *mit*$^+$/*mit*$^-$ – general term for the allelic forms of mitochondrial genes coding for the enzymes of internal mitochondrial membrane; *OXI1*, *OXI2*, *OXI3* – mitochondrial loci coding for the second, third and first cytochrome c oxidase subunits, respectively; *oxi1*, *oxi2*, *oxi3* mutations in the above mentioned loci; *BOX* – mitochondrial DNA region controlling both cytochrome b and cytochrome c oxidase; C^R, E^R – mutations in *RIB1* and *RIB3* mitochondrial loci, respectively.

3. BASIC PROCEDURE AND RESULTS

The consecutive steps of construction of isomitochondrial and isonuclear derivatives of the 777-3A strain are given in Fig. 1.

(i) *Isolation of revertants of the* op1 *mutation*

Cells of the 777-3A strain were plated on N3 medium at a concentration of 5×10^7 cells per plate. Colonies of spontaneous phenotypic revertants of the *op1* mutation appeared with a frequency of $0{\cdot}5–1 \times 10^{-9}$. Eight such revertants were isolated and subcloned twice on N3 medium.

Three of the phenotypic revertants seemed to result from a suppressor mutation unlinked with the *op1* mutant gene, since in the haploid progeny of their crosses with the standard *OP1* strain IL8-8D about 25% of ascospores did not grow on N3 medium. Moreover, the three types of tetrads 4:0 (growing : not growing on N3), 2:2 and 3:1 corresponding to PD, NPD and T classes of tetrads were observed, PD and NPD classes being approximately equal.

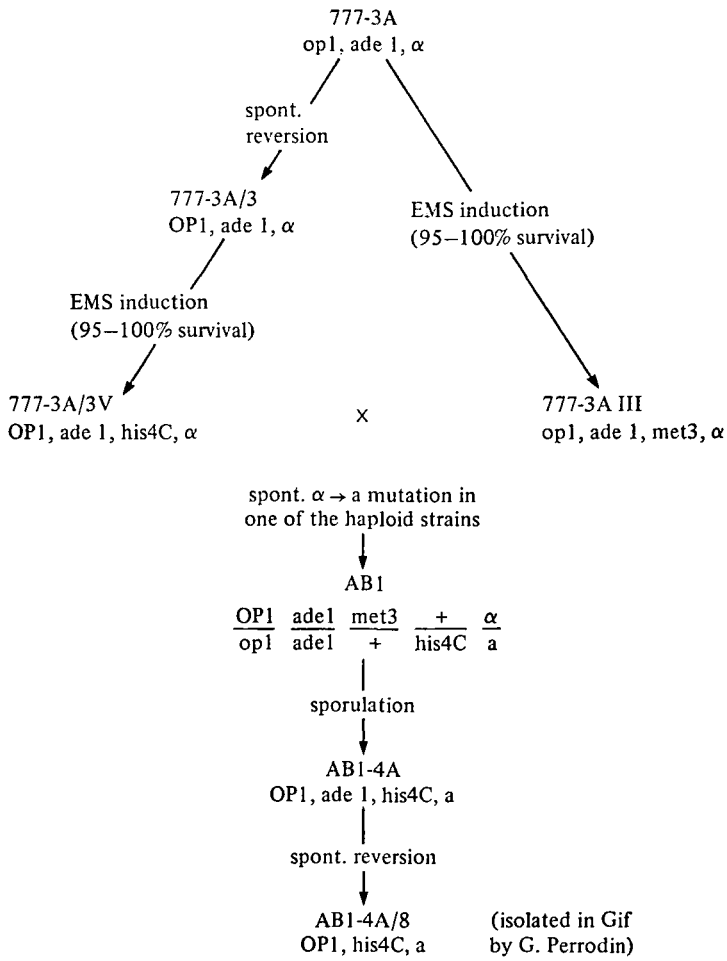

Fig. 1. Consecutive steps in construction of isomitochondrial and isonuclear strains. The mitochondrial genotype of the original 777-3A strain and all its derivatives was $\rho^+\omega^+$.

In the progeny of the other 5 phenotypic revertants crossed with the IL8-8D strain either only 4:0 tetrads, or 4:0 and some infrequent 3:1 tetrads were found. The vegetative progeny of each ascospore not capable of growth on N3 was crossed with *op1*ρ^+ strain (LK1-4C or 777-3A) and with *OP1*ρ^0 strain (SP4/1 or DT15/1). Only the diploid progeny from crosses with the *OP1*ρ^0 strain did not grow on N3 medium. This indicated that all the ascospores not growing on N3 medium were not the *op1* segregants, but spontaneous mitochondrial ρ^- mutants. Therefore, the revertants resulted either from true back mutation or from a suppressor mutation closely linked with the *op1* mutant gene.

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From the latter group strain 777-3A/3 was chosen for further work as it grew on N3 medium at a rate similar to that of the standard wild-type strain. In the cross of 777-3A/3 with the standard *OP1* strain IL8-8D, out of 14 tetrads analysed one ascospore did not grow on N3 medium owing to ρ^- mutation. Similarly to other revertants of the *op1* mutation, strain 777-3A/3 showed a rather high frequency of ρ^- spontaneous mutations. It had about 10 % of ρ^- cells after about 20 cell generations in glucose complete medium, but only about 1 % in glucose minimal medium.

(ii) *Isolation of new auxotrophs in 777-3A and 777-3A/3 strains*

The original 777-3A strain and the 777-3A/3 strain were treated with EMS (ethylmethanesulphonate) to induce new auxotrophic mutations. This was done as follows. The 777-3A strain was grown for 24 h on solid NO medium, while the 777-3A/3 strain for 48 h on N3 medium. Then, the cells were suspended in 0·1 M phosphate buffer, pH 7·0, at a density of 1×10^8 cells/ml and treated either with 2 % EMS for 45 min or with 3 % EMS for 15 min. The reaction was stopped by 50-fold dilution of the treated cells with 6 % sodium thiosulphate. The cells were then centrifuged, washed with saline, and after proper dilution plated on NO medium. In both strains, both types of treatments gave 95–100 % survival. Colonies grown on NO medium were replica-plated on GOade medium. In each of the treated strains 12 new auxotrophic mutants were isolated and the new requirements were determined by an auxonography test.

(iii) *Isolation of α/a diploids*

The newly isolated auxotrophs from strain 777-3A were mixed with those isolated from 777-3A/3 in all possible combinations as regards complementarity of nutritional requirements: two drops of each strain containing about 5×10^6 cells were placed on GOade medium and incubated for 3 days at 30 °C. In some combinations infrequent putative zygotic clones appeared. They were harvested and subcloned on GOade medium. Out of several strains obtained in such a way, only one sporulated normally and produced ascospores that germinated well. The diploid arose owing to spontaneous mutation from α to a in one of the two haploid strains and had the following genotype:

$$\frac{OP1}{op1}\,\frac{ade1}{ade1}\,\frac{met3}{+}\,\frac{+}{his4C}\,\frac{\alpha}{a}\,[\rho^+\,\omega^+].$$

The loci in which new *his* and *met* mutations occurred were determined by complementation tests. The diploid shows normal growth on N3 medium and tolerates ρ^- mutations. Thus, if the reversion of the *op1* mutation was due to a closely linked suppressor mutation (i.e. if the diploid had in fact rather a (op1 SUP)/(op1 sup) than OP1/op1 genotype), the suppressor would be fully dominant over the double dose of the *op1* mutation.

(iv) *Isolation of haploid strains*

The diploid described above was sporulated on VB medium and several tetrads were isolated giving rise to a set of isomitochondrial and isonuclear strains differing only in histidine and methionine requirements, mating type and *OP1* versus *op1* mutation. In one of the strains AB1-4A (*OP1 ade1 met3 a*) a spontaneous adenine prototroph (AB1-4A/8) was isolated (by G. Perrodin in Gif), so that it can be easily crossed with the original 777-3A strain and other adenine-requiring isonuclear and isomitochondrial strains.

4. DISCUSSION

To ensure that the strains constructed were really isomitochondrial and isonuclear, spontaneous mutations were isolated in all the steps where positive selection of mutants

was possible. The cells were mutagenized only for the induction of auxotrophic mutations in strains 777-3A and 777-3A/3, and at this stage only was the chance of occurrence of uncontrolled nuclear mutations increased. At as low doses as those used here, EMS does not seem to be mutagenic towards mitochondrial DNA (Polakowska, personal communication). Thus, the strains constructed can be considered with a good approximation as being actually isomitochondrial and isonuclear. Their construction is useful not only for genetic analysis of *OXI1* and *OXI2* loci, but also of other *mit*$^-$ loci especially *BOX* and *OXI3*, as many mutants of these loci were isolated in the 777-3A strain.

It was observed that the diploids formed between α and a strains carrying different auxotrophic mutations, but otherwise with the same 777-3A background, like those obtained from strains with different genetic backgrounds, have higher growth rates than the haploid parental strains and do not form clumps. This suggests that fast growth and the lack of clump formation in diploid strains is not connected with the heterozygous state of many genes.

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REFERENCES

Biliński, T., Łukaszkiewicz, J. & Śledziewski, A. (1978). Demonstration of anaerobic catalase synthesis in the *cz1* mutant of *Saccharomyces cerevisiae*. *Biochemical and Biophysical Research Communications* **83**, 1225–1233.

Borst, P. & Grivell, L. A. (1978). The mitochondrial genome of yeast. *Cell*. **15**, 705–723.

Coen, D., Deutsch, J., Netter, P., Petrochilo, E. & Slonimski, P. P. (1970). Mitochondrial genetics. I. Methodology and phenomenology. In *Control of Organelle Development* (ed. P. L. Miller), pp. 449–496. Cambridge University Press.

Dujon, B., Bolotin-fukuhara, M., Coen, D., Deutsch, J., Netter, P., Slonimski, P. P. & Weill, L. (1976). Mitochondrial genetics. XI. Mutations at the mitochondrial locus ω affecting the recombination of mitochondrial genes in *Saccharomyces cerevisiae*. *Molecular and General Genetics* **143**, 131–165.

Galzy. P. & Slonimski, P. P. (1957). Chimie biologique-variations physiologiques de la levure au cours de la croissance sur l'acide lactique comme seule source de carbone. *Comptes Rendus de l'Académie des Sciences* (*Paris*) **245**, 2423–2426.

Kotylak, Z. & Slonimski, P. P. (1977). Mitochondrial mutants isolated by a new screening method based upon the use of the nuclear mutation *op1*. In *Mitochondria 1977: Genetics and Biogenesis of Mitochondria* (ed. W. Bandlow, R. J. Schweyen, K. Wolf & F. Kaudewitz), pp. 83–89. Berlin: De Gruyter.

Kováč, L., Lachowicz, T. M. & Slonimski, P. P. (1967). Biochemical genetics of oxidative phosphorylation. *Science* **158**, 1564–1567.

Kováčová, V., Irmlerová, J. & Kováč, L. (1968). Oxidative phosphorylation in yeast. IV. Combination of a nuclear mutation affecting oxidative phosphorylation with cytoplasmic mutation to respiratory deficiency. *Biochimica et Biophysica Acta* (*Amsterdam*) **162**, 157–163.

Mortimer, R. K. & Hawthorne, D. C. (1969). In the *Yeast* (ed. A. H. Rose and S. J. Harrison), pp. 386–460. London, New York: Academic Press.