Effects of the kar Gene on Cytoplasmic Mixing and Mitochondrial Genome Suppressiveness, and Consequences for Cytoduction of petite DNA in *Saccharomyces cerevisiae*

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Summary. During a series of cytoduction experiments to transfer *Saccharomyces cerevisiae* mitochondrial genomes from one nuclear background to another, using the karl-1 nuclear fusion mutation, one of the five petite genomes used proved difficult to transfer. This genome, ρ−F13, was highly suppressive (90%) in its original nuclear background. Molecular and genetic studies on the putative karl-1 ρ−F13 cytoductant were done to discover the nature of this difficulty. They showed that while the ρ−F13 was maintained in a karl-1 background, zygotes from a mating with a ρ0 strain showed poor cytoplasmic mixing and therefore inefficient ρ−F13 DNA transfer into first zygotic buds. This also caused a reduction of ρ−F13 suppressiveness to 20–30% in crosses with different ρ+ strains. The effect was genome specific since another highly suppressive petite in the karl-1 background did not show suppressiveness reduction when crossed to ρ+. The nature of suppressiveness modulation is discussed. Since the ρ−F13 genome was eventually transferred using a modification of the original scheme, the problems were not caused by the inability of the acceptor nuclear background to maintain the ρ−F13 genome.

Key words: *Saccharomyces* – Cytoduction – Mitochondrial genomes – Suppressiveness

Introduction

Mutant (*petite* or ρ−) mitochondrial genomes in the yeast *Saccharomyces cerevisiae* can be classified according to their transmission patterns in matings with cells containing a wild type (grande or ρ+) mitochondrial genome (Ephrussi et al. 1955). At the level of transmission genetics, their identification is based upon the number of petite zygotic colonies produced from a ρ− by ρ+ mating. The parental petite is termed neutral if the frequency of petite zygotic colonies produced is no higher than the frequency of spontaneous petite mutants in the ρ+ parent. Petite production above this frequency is used to classify the petite parent as suppressive and the degree of suppressiveness of a particular ρ− is defined by a cross with a specific ρ+ strain (Ephrussi and Grandchamp 1965). If a particular ρ− × ρ+ cross produces a high percentage of petite zygotic colonies, the petite parent is defined as highly suppressive. According to this terminology a neutral petite shows 0% suppressiveness and a petite without mitochondrial DNA (ρ0) is neutral.

To control for nuclear effects on mitochondrial properties it is often necessary to study a series of isonuclear strains which differ only in their mitochondrial genomes. Such strains could be produced (i) by initially selecting mitochondrial mutations in a single nuclear background; (ii) by fusing ρ0 cell protoplasts of a particular strain with a series of isolated mitochondria containing different types of mitochondrial DNA molecules (Ortega Ruiz 1977; Gunge and Sakaguchi 1978); (iii) by transforming the same ρ0 strain with different types of mitochondrial DNA molecules (this procedure is hypothetical at present); or (iv) by using the karl-1 nuclear fusion mutation to produce parental-type haploid products containing different mitochondrial genomes via cytoduction (Conde and Fink 1976; Nagley and Linnane 1978; Lancashire and Mattoon 1979). For my initial purpose, I chose the last procedure for producing a set of isonuclear α mating type strains bearing well characterized mitochondrial neutral and suppressive genomes. These isonuclear strains were produced to study the transmission of mitochondrial DNA during yeast mating.
Several mitochondrial genomes were successfully and uneventfully transferred by cytoduction. A surprising problem arose with a highly suppressive petite genome designated ρ−F13. It was very difficult to transmit this genome through the karl-1 haploid intermediate and into haploid progeny containing the desired (a-4) nuclear background. This was surprising since current molecular models for suppressiveness theorize that such mitochondrial genomes retain a reiterated DNA sequence associated with replication (a putative replication origin) (deZameroczy et al. 1979; Blanc and Dujon 1980). Consequently, theory predicts that mitochondrial genomes with such a reiterated sequence can out-replicate grande genomes in crosses, resulting in a high frequency of petite genome transmission to the progeny and the expression of suppressiveness. If all suppressive molecules had this capability, it seemed curious that a highly suppressive genome would not be readily transmitted to progeny. A study was undertaken to characterize more fully the reasons for this inefficient genome transfer. The study focused on a careful analysis of the putative karl-1 ρ−F13 intermediate in the cytoduction scheme used. Its ability to maintain and transmit the ρ−F13 mitochondrial genome in classical suppressiveness tests was studied. The results of these experiments are described in this paper.

Materials and Methods

Yeasts. Five a strains were used as sources of mitochondrial genomes for the cytoduction experiments in Fig. 1: IL8-8C/E41 ρ− (neutral petite), IL8-8C/E41/S1 ρ− (neutral petite), IL8-8C/F13 ρ− (90% suppressive) all obtained from P. Slonimski, and described elsewhere (Sena et al. 1981; Locker and Rabinowitz 1976), a spontaneous suppressive isolated in my laboratory from the IL8-8C/E41 designated IL-8-8C/E41-3-13 (50–60% suppressive); and a highly suppressive petite strain ρ−8-3 (98% suppressive) isolated after ethidium bromide treatment of the original a-4 ρ− parent (Sena et al. 1981). The suppressiveness of each strain was determined by the classical zygotic colony assay with the same grande a mating type tester strain 7b ρ+. The ρ−8-3 strain carrying the nuclear background. Only the p-F13 mitochondrial genome proved difficult to obtain in the a-4 progeny of the cross between karl-1 and KAR1 often produce haploid products containing one or the other haploid parental nucleus and cytoplasmic components from one or the other or both parents (Conde and Finke 1976; Nagley and Linnane 1978; Lancashire and Matoon 1979). If haploid parental nuclei become associated with cytoplasmic components put into the zygote from the other parent the resulting haploid is termed a cytoductant (Zacharov and Yarovoy 1977). Diploid nuclear formation occurs when parental nuclei fuse. This is more frequent in some crosses than others. Often a transient heterokaryotic zygote is formed which can upon bud initiation segregate haploid, diploid and heterokaryotic products. Using the crossing procedure outlined in Fig. 1 the ρ−E41, ρ−E41/S1, ρ−E41-3-13 and the ρ−8-3 petite mitochondrial genomes could be successfully transferred from their original nuclear backgrounds through the karl-1 nuclear background and into the a-4 nuclear background. Only the ρ−F13 mitochondrial genome proved difficult to obtain in the a-4 progeny of the cross between karl-1 ρ−F13 and a-4 ρ0 (stage 2 cross 2). All petite first zygotic buds from initial stage 2 cross 2 matings produced clones which were neutral in matings with a standard ρ+ tester (7b). One a bud product, also neutral, was analyzed at the DNA level and turned out to be ρ0.

Results

As stated in the Introduction, isonuclear strains containing different mitochondrial genomes can be readily obtained by using a parent bearing the karl-1 nuclear fusion mutation in a crossing protocol. Zygotes derived from crosses between karl-1 and KAR1 often produce haploid products containing one or the other haploid parental nucleus and cytoplasmic components from one or the other or both parents (Conde and Finke 1976; Nagley and Linnane 1978; Lancashire and Matoon 1979). Standard media for yeast culture maintenance and nuclear and mitochondrial genetic analysis were used, as described elsewhere (Sena et al. 1981). Medium for scoring inositol requirements contained 16.7 g Vitamin Free Yeast Base (Difco), 20 g agar (Difco), 1 ml vitamin mixture (0.2 mg biotin, 40 mg pantothenate, 40 mg pyridoxine/100 ml) per liter and amino acid additions (Sena et al. 1981) as needed. Enriched medium is designated as YEP, minimal drop out medium as YNB and medium for scoring mitochondrial markers and respiratory sufficiency as N3.

Basic Cytoduction Procedure. To produce a series of excellently mating isonuclear strains containing specific mitochondrial genomes, mitochondrial DNA was transferred into an a-4 LEU1 trp4 petite nuclear background. The procedure involved crosses with several a ρ− strains and the same a karl-1 ρ− (genotype: a his3-5 inol ino4 karl-1 ρ−), followed by matings of a karl-1 ρ− haploid progeny with a-4 ρ0. The series of crosses is depicted in Fig. 1. For each cross unbudded cells of the proper genotype were mated by micromanipulation on solidified YEP agar medium as described previously (Sena et al. 1981). First and, sometimes, second zygotic buds were removed from the resulting zygotes. All plates were incubated at 30°C between manipulations and thereafter. Haploid and diploid products were analyzed by standard genetic techniques. Molecular analysis of mitochondrial DNA composition was carried out as described elsewhere (Sena et al. 1976).