Genetic Analysis of an Osmotic Sensitive Saccharomyces cerevisiae Mutant

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Summary. The genetic analysis of VY1160 sorbitol dependent, osmotic sensitive yeast mutant led to the identification of three different nuclear recessive mutations. Two of them, designated sorb- and tsl are closely linked to one another. The mutation sorb- determines the lysis, while the mutation tsl increases the ability for lysis of the sorbitol dependent cells. The third mutation ts2 segregates independently from the other two and confers the sensitivity of VY1160 mutant cells towards rifampicin.

Introduction

The isolation and partial characterization of osmotic sensitive yeast mutants have been reported (Hartwell - unpublished results cited in Mortimer and Hawthorne, 1973; Cabib and Duran, 1975; Le Din Long, 1977). However, the isolated mutants were more or less unstable and lysed only after prolonged incubation at nonpermissive temperatures. Because of these disadvantages, these mutants can not be used for molecular biological experiments.

The isolation of stable osmotic sensitive mutants of Saccharomyces cerevisiae has been reported (Venkov et al., 1974). The mutants grow exponentially at 30 °C in media supplemented with sorbitol as osmotic stabilizer. Suspended in buffers lacking the osmotic stabilizer, the cells lyse immediately releasing 60-80% of their cellular contents, this phenomenon being observed even at 4 °C. Electron microscopic investigations have revealed that under the conditions of the osmotic shock, local ruptures appear in the cell wall through which different subcellular components flow out (Mateeva et al., 1976). The isolated osmotic sensitive mutants are also sensitive to higher temperature (37 °C) and display an increased susceptibility to different antibiotics like actinomycin D (Waltschewa et al., 1976), toyocamycin (Venkov et al., 1977) and rifampicin (Venkov et al., 1975).

Here we report the results obtained by the genetic analysis of VY1160 stable osmotic sensitive yeast mutant. The strain proved to carry three different nuclear recessive mutations. Two of them, sorb- and tsl, are located close to one another. The sorb- mutation determines the cell-lysis, while tsl mutation only increases the percentage of lysed cells in the sorbitol dependent strains. The third mutation ts2, determines the sensitivity to rifampicin of VY1160 mutant cells.

Materials and Methods

Strains and Media. The following Saccharomyces cerevisiae strains have been used: A364 a, adel, ura1, gai1, trpl, his7, lys2, trpl (from the collection of Dr L. Hartwell, University of Washington); S288C a, gal2 (from the collection of Dr G. Fink, Cornell University); IPG-104 a, his8 (from the collection of Dr I. Zakharov, Leningrad Nuclear Physics Institute). VY1160 a, is a sorbitol dependent, osmotic sensitive, respiratory deficient mutant, isolated from strain S288C (Venkov et al., 1974). The mutant also fails to grow at 37 °C and is rifampicin-sensitive and auxothrophic for several amino acids. The YPD (rich), SD (minimal), presporulation and sporulation media (Sherman et al., 1970) and the YM-5 medium (Hartwell, 1967) have been supplemented with 10% of sorbitol as osmotic stabilizer.

Genetic Methods. Standard techniques have been used to obtain hybrids by crossing auxothrophs. The distribution of the markers tested has been studied by random spore and tetrad analysis (Zakharov et al., 1976).

Cell-Lysis Measurements. The lysis in water of the osmotic sensitive cells was determined as described previously (Venkov et al., 1974). In cases of measuring the lysis after cultivation at 37 °C, the cells were grown at the permissive temperature (30 °C) to mid-exponential phase and then shifted to 37 °C. Lysis of the cells was determined after incubation for 3.5 h at the restrictive temperature.

Rifampicin Susceptibility Determinations. The sensitivity towards rifampicin was tested in two different ways. Cells were replica-
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plated on solid YM-5 medium with and without 250 μg/ml of rifampicin and the degree of cell-growth determined after 2-3 days of incubation at 30°C. Besides this, each strain was grown in liquid YM-5 medium at 30°C to mid-exponential phase and the cells were labelled with 0.2 μCi/ml of [³H]-uracil (58 mCi/mM, The Radiochemical Centre Amersham, U.K.). The incorporation of the label into RNA was followed in presence or absence of 100 μg/ml of rifampicin. Strains, sensitive to rifampicin do not grow on solid medium containing rifampicin and incorporate in the presence of the antibiotic no more than 50% of the label as compared with the control.

Results

Genetic Analysis of Hybrids VY1160 × IPG-104. The diploids obtained by crossing the strain VY1160 with either IPG-104 or A364 were prototrophic and had a high ability for sporulation. In these experiments only the respiratory deficiency, temperature sensitivity and sorbitol dependence were tested. The capability for lysis and sensitivity towards rifampicin were studied in the later stages of the analysis.

Random spore analysis of the haploids from the cross VY1160 × IPG-104 displayed a complicated segregation of the markers tested. The diploids themselves were not respiratory deficient. The transmission of the respiratory deficiency in the haploid spores was characteristic for a mitochondrial mutation. As the presence or absence of this particular mutation could not be connected with any of the markers of interest, the respiratory deficiency was not studied further.

The segregation of temperature sensitivity (ts⁻) and sorbitol dependence (sorb⁻) is presented in Fig. 1. The segregation of sorb⁻ character in spores was compatible with the assumption that one nuclear mutation was responsible for this character in VY1160 strain. The smaller number of sorb⁻ segregants is most probably a result of the poorer viability of ascospores carrying the sorb⁻ mutation, a fact also supported by the results of the tetrad analysis. All sorb⁻ segregants were also temperature sensitive. On the basis of these results it can be assumed that the temperature sensitivity is one of the pleiotropic effects of the sorb⁻ mutation.

The analysis of the sorbitol independent (sorb⁺) ascospores displayed 1:1 segregation of the ts⁻ character. These results suggested the presence of another ts mutation which segregates independently from the sorb⁻ character.

The tetrad analysis of the hybrid VY1160 × IPG-104 was hampered, because of the poor viability of the ascospores. In the four full tetrades (out of 12 dissected) the sorb⁻ character showed mendelian segregation. All sorb⁻ haploids turned out to be ts⁻ too.

Genetic Analysis of Segregants in Second Set of Crosses with IPG-104. Hybrids obtained by crossing segregants: sorb⁺ts⁺, sorb⁻ts⁻ and sorb⁺ts⁻ with IPG-104 were tested (at least 5 diploids of each type).

Spore analysis of the hybrid sorb⁺ts⁻ × IPG-104 showed the absence of both sorb⁻ and ts⁻ segregants. In the progeny of sorb⁺ts⁻ × IPG-104 cross segregation of the sorb⁻ and ts⁻ characters was found (Fig. 2). In this particular case the segregation of sorb⁻ was 1:1 which is in full agreement with the assertion that this character is determined by one nuclear mutation. However, in contrast to the results discussed previously (Fig. 1), sorb⁺ts⁻ and sorb⁻ts⁻ recombinants were found in these experiments. All sorb⁻ts⁻ haploids were again backcrossed with a wild type strain. In the progeny approximately 1:1 segregation of sorb⁺:sorb⁻ alleles was found, but no ts⁻ segregants were recovered.

These results show that the temperature sensitivity is not one of the pleiotropic effects of the mutation that determines the sorbitol dependence of the VY1160 strain. The ts⁻ character is determined itself by a separate mutation, which probably is localized close to the mutant gene for the sorb⁻ character.

The analysis of the hybrid sorb⁺ts⁻ × IPG-104 showed in all cases a 1:1 segregation of ts⁻:ts⁺ (a total of 53 ts⁻:61ts⁺). In this way another nuclear recessive mutation was identified, different from that found in analysis of VY1160 × IPG-104 cross. Following data support this conclusion. First of all, in the preliminary analysis of the VY1160 × IPG-104 cross 57 ts⁻ and 30 ts⁺ segregants were found, a result