The Effect of Liquid Holding on Chemical
Induced Lethality and Mitotic Gene Conversion
in *Saccharomyces cerevisiae*

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Summary. Mitotic gene conversion was induced with a variety of chemical mutagens in a double heteroallelic strain of *Saccharomyces cerevisiae*. Cells were treated with various mutagens and plated immediately onto selective and nonselective growth medium or else they were subject before plating to liquid holding in buffer for various lengths of time. In respiratory competent cells liquid holding caused a decrease in lethality and in conversion frequencies. Respiratory deficient cells, unable to use a non-fermentable substrate as an energy source, behaved different. Untreated cells started to die in buffer after two days of storage, and moreover, there was a considerable increase in potential convertants i.e. cells giving rise to gene convertants when plated on selective growth media. Respiratory deficient cells treated with various chemical mutagens were still more sensitive to liquid holding. After low, sublethal doses cells started to die after one day of liquid holding already and when plated on media selective for convertants, showed an increasing frequency of gene convertants. Addition of very low concentrations of glucose to the liquid holding buffer postponed the lethal and convertogenic effects. Higher concentrations of glucose completely abolished sensitivity to liquid holding-induced lethality and genetic alterations. The results are interpreted to mean that in respiratory deficient cells no repair activities are possible during liquid holding due to a lack of chemical energy. The absence of repair activities leads to an accumulation of spontaneous lethal damage and genetic alterations which are expressed as gene conversion when an energy source becomes available. Such a repairless condition causes an increased sensitivity to genetically active agents, and provides a useful system to detect genetic effects of slowly reacting agents.

Introduction

The discovery of enzyme systems involved in the repair of genetic damage raises the question for the extent of genetic damage which can be completely neutralized by these systems. This question is of particular relevance since there might be metabolic conditions which prevent repair systems from operating and since there exist agents like caffeine interfering with repair systems. If caffeine is added to bacteria treated with sublethal doses of ultraviolet light there is a considerable mutagenic effect to be observed which cannot be detected in the absence of caffeine (SHANKEL and WYSS, 1961; DONESON and SHANKEL, 1964).

In the yeast *Saccharomyces cerevisiae* there are known cytoplasmic respiratory deficient mutants which have lost the ability to perform an oxidative metabolism and which are restricted to meet their requirements for chemical energy exclusively by glycolysis. If such a mutant strain is kept in a buffer free of glucose, cells can only metabolise intracellular pools of glucose or break down glycogen reserves. After consumption of these supplies the cells are unable to perform any energy requiring reactions. Repair processes involve excision of damaged
DNA base sequences (Setlow and Carrier, 1964; Boyce and Howard-Flanders, 1964) and subsequent repair replication to fill the gaps caused by excision of base sequences damaged not only by ultraviolet light but also by chemicals (Pettijohn and Hanawalt, 1964; Cerda-Olmedo and Hanawalt, 1967). At least some of these reactions depend on the availability of chemical energy, and consequently storing of respiratory deficient cells in media free of glucose should reveal damage otherwise repaired by energy requiring systems.

This expectation was borne out. Storing of cells treated with low doses of mutagens resulted in the appearance of genetic alterations, which were otherwise abolished upon immediate exposure of the same cells to glucose medium.

Mitotic gene conversion was chosen as the test system for genetic damage because it allowed to work with selective methods by scoring prototrophic cells arising by gene conversion from a population of auxotrophs. This methodic advantage of gene conversion is accompanied by a possible theoretical advantage over reserve mutation systems. In extensive experiments with numerous mutagens no exclusive specific response of a given heteroallelic combination to only a few mutagens was to be observed (Zimmermann and Schwager, 1967 and unpublished results). Such specificities were frequently found in reverse mutation experiments by numerous workers in numerous organisms. Moreover, it was found that the influence of repair processes on reverse mutations induced in Schizosaccharomyces was specific for certain inducing mutagens (Clabke, 1968).

Material and Methods

The strain used was the diploid D 4 which was heteroallelic at the loci \( ad \) and \( tr \) and has been described in details by Zimmermann and Schwager (1967). The alleles \( ad_1 \), \( ad_2 \), \( tr_{r-2} \), \( tr_{r-12} \) and \( tr_{r-37} \) were kindly provided by Dr. T. R. Manney, Cleveland, Ohio, USA (Manney, 1964). A respiratory deficient derivative of this strain was selected. Cells were grown in 5 ml liquid YEP (1% Difco yeast extract, 2% Difco bacto peptone, 2% glucose) in cotton plugged test tubes on a rotary shaker at room temperature (20–24°C). Upon reaching stationary phase, cultures were stored in a refrigerator (4°C) for 3–4 days. Such cells were washed and treated with freshly prepared buffered solutions of the mutagens for a given time and then washed free of the agent. Washed cells were either plated immediately on synthetic media (Romani, 1956) containing glucose as a carbon source or else were incubated for different times in 0.1 M phosphate buffer pH 7.0 at 25°C before plating.

Results

I. Respiratory Competent Cells

In a first series of experiments normal respiratory competent cells of strain D 4 were treated with nitrous acid.

The doses used were chosen in a way to allow for investigating the effect of nitrous acid over a range of a barely detectable reduction of the ability to form colonies to a strong effect giving only 29.2% survival. In Table 1 it can be clearly seen that after treatment with lethal doses liquidholding of respiratory competent cells counteracts nitrous acid induced lethality.

Mitotic gene conversion was studied at the loci \( tr \) and \( ad \). It can be seen that in an untreated control population the frequency of convertant cells for these two loci remained constant for two days (Table 1). On the third day the frequency for convertants at the \( tr \) locus started to increase whereas convertants...