Nucleic Acid Metabolism in Yeast

I. Inhibition of RNA and DNA Synthesis by High Concentrations of Exogenous Deoxythymidine 5'-monophosphate in 5'-dTMP Low Requiring Strains

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Summary. The three haploid yeast strains T2tmpl-3, T2tmpl-1, and T6tmpl-51 auxotrophic for 5'-dTMP differ in their requirement for thymidylate: 72, 16, and 3 μg 5'-dTMP/ml will restore optimal growth, respectively. Thymidylate low requirement in strain T2tmpl-1 and T6tmpl-51 is termed tlrA and tlrC, respectively. When the growth medium is made 5 x 10^-4 M for 5'-dTMP only strain T6tmpl-51 is severely inhibited in RNA and DNA synthesis. This inhibition is reversible after removal of excessive 5'-dTMP. The inhibitory characteristic is in marked contrast to "thymineless death" due to the lack of 5'-dTMP in strain T6tmpl-51 where only DNA synthesis stops while RNA synthesis continues. The inhibitory effect of 5 x 10^-4 M 5'-dTMP is not due to the 5'-dTMP auxotrophy but to the thymidylate low requiring character (tlrC) in strain T6tmpl-51. The arrest of RNA and DNA synthesis by high concentrations of exogenous 5'-dTMP suggests a regulatory role of either the monoo or triphosphate on nucleoside or nucleotide biosynthesis in yeast.

Introduction

Recently methods for the DNA-specific labelling of Saccharomyces cerevisiae have been developed. Starting with a strain that could utilize 5'-dTMP as a precursor for its DNA synthesis (Jannsen, Lochmann and Laskowski, 1968; Jannsen, Lochmann and Megnet, 1970; Brendel and Haynes, 1972, 1973; Fäth and Brendel, 1974) the efficiency of utilization of 5'-dTMP was greatly enhanced by selective screening of 5'-dTMP low requiring mutants (Fäth, Brendel, Laskowski and Lehmann-Brauns, 1974; Brendel, Fäth and Laskowski, in press). The isolation of 5'-dTMP auxotrophic mutants greatly facilitated this screening (Fäth et al., 1974; Brendel and Fäth, 1974), and lead to yeast strains that can take up, and incorporate into their DNA, 20 to 50 per cent of the exogenously offered nucleotide (Fäth and Brendel, in press).

This report deals with the inhibitory effects of high concentrations of 5'-dTMP on yeast mutants with low requirement for this nucleotide.

Materials and Methods

Strains. The yeast strains employed are given in Table 1. All of them are derived from haploid strain 211-1aM, T-series (Laskowski and Lehmann-Brauns, 1973). The designations tlrA and tlrC stand for different abilities of the corresponding strains to utilize 5'-dTMP. As no genetic analysis is possible at the moment - all tlr strains have lost their mating type and cannot be crossed successfully - the tlr character cannot be related to a nuclear gene and thus is written in brackets.

Standard Growth Conditions. Strains were grown in medium N with appropriate supplement of 5'-dTMP (Frith and Brendel, 1974; Brendel and Langjahr, 1974). Media were supplemented with 5'-dTMP (Merck) from a 10^-2 M stock solution in distilled water.

Thymineless Death, DNA and RNA Synthesis. The procedures were essentially as described by Brendel and Haynes (1973) and Brendel and Langjahr (1974). Removal of high concentrations of 5'-dTMP was achieved by three rapid centrifugations in an Eppendorf centrifuge and washings in medium N. After the final run cells were resuspended in medium supplemented with the appropriate amount of 5'-dTMP.

Assay of Radioactivity. This was essentially done as described by Brendel and Haynes (1973) and Fäth and Brendel (1974).

Table 1. Description of yeast strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>Complete name and genotype</th>
<th>5'-dTMP requirement</th>
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<tbody>
<tr>
<td>T2tmpl-3</td>
<td>α ilc2 typ1 tmp1-3 rho-</td>
<td>72 μg/ml</td>
</tr>
<tr>
<td>T2tmpl-1</td>
<td>(x) ilc2 typ1 tmp1-1 rho- (tlrA)</td>
<td>16 μg/ml</td>
</tr>
<tr>
<td>T6tmpl-51</td>
<td>(x) ilc2 typ1 tmp1-51 rho- (tlrC)</td>
<td>3 μg/ml</td>
</tr>
<tr>
<td>16-425</td>
<td>(x) ilc2 typ1 TMP rho- (tlrC)</td>
<td>none*</td>
</tr>
</tbody>
</table>

* In the presence of aminopterin and sulfanilamide this strain requires 3 μg/ml 5'-dTMP for growth (Fäth and Brendel, in the press).
Results

The dependence of growth on 5'-dTMP for three \textit{tmp} strains is shown in Fig. 1. Strains \textit{T6tmp1-51}, \textit{T2tmp1-1}, and \textit{T2tmp1-3} exhibit optimal growth in medium \textit{N} supplemented with 3, 16, and 72 \mu g 5'-dTMP/ml, respectively. At concentrations higher than needed for the optimal supplementation of the auxotrophy, 5'-dTMP becomes inhibitory. Sensitivity to high concentrations of 5'-dTMP is most pronounced in strain \textit{T6tmp1-51} which, at approximately 200 \mu g 5'-dTMP/ml and 24 hours of incubation shows no growth and thus the same response as if no 5'-dTMP were added to the medium (Fig. 1). The strains \textit{T2tmp1-1} and \textit{T2tmp1-3}, though exhibiting different requirement of 5'-dTMP for optimal growth seem to be of similar resistance to high concentrations of the nucleotide. At 600 \mu g 5'-dTMP/ml they still grow to about half the optimal cell titer (Fig. 1).

The effects of high, optimal, and zero supplement of 5'-dTMP on growth of strain \textit{T6tmp1-51} is shown in Fig. 2. Cell numbers stay constant when cultures of exponentially growing cells of \textit{T6tmp1-51} are either deprived of 5'-dTMP or supplemented with 180 \mu g 5'-dTMP/ml (5 \times 10^{-4} M) (Fig. 2a). The effect on the morphology of the cells, however, is quite different.