SPECIFIC INCORPORATION OF EXOGENOUS THYMIDINE MONOPHOSPHATE INTO DNA IN *SACCHAROMYCES CEREVISIAE*

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1. Introduction

Fungi incorporate exogenous thymidine very inefficiently [1-4]. When labelled thymidine is administered, the label is found in both RNA and DNA [3]. These results which are a consequence of the lack of thymidine kinase and a pathway which converts thymine to uracil [3,4] preclude the use of radioactive thymidine as a means of specifically labelling newly synthesized DNA in yeast and other fungi. However, the rate and the specificity of labelling of DNA in yeast can be improved when ^{14}C-thymidine is replaced by ^{14}C-thymidine-5-monophosphate (TMP) [2].

In this paper we show that cells of the strain 211 of *Saccharomyces cerevisiae* [5] incorporate exogenous 2-^{14}C-TMP specifically into DNA.

2. Experimental

The strain 211 of *Saccharomyces cerevisiae* [5] was kindly supplied by Dr. W. Laskowski. The cells used for the isolation of labelled DNA were grown for 48 hr, at 30°, in 500 ml shake culture in a medium (YPG) containing glucose 2%, peptone 1%, and yeast extract 0.5%. After autoclaving, 50μCi of 2-^{14}C-TMP, 43.6 mCi/mmole, from New England Nuclear Corporation was added.

DNA was prepared from cells washed twice with 50 mM phosphate buffer pH 4.5, in the following manner: the cells were broken with glass beads in an MSK homogenizer (Braun, Melsungen, Germany). After adding sodium dodecyl sulfate to a final concentration of 2%, the homogenate was kept for 10 min at 60° and sodium perchlorate was added to a final concentration of 1 M. The proteins were denatured by adding an equal volume of chloroform-isoamylalcohol (25:1) and shaking for 30 min. The emulsion was broken by centrifugation and the upper layer, containing the nucleic acids, was treated with a mixture of pancreatic RNAase (freed from DNAase by heating), 0.1 mg/ml, and T_1 ribonuclease (freed from DNAase by heating), 20 units/ml, and incubated for one hour at 37°. Solid sodium chloride was added to a final concentration of 1 M and then, the solution was treated with pronase (freed from DNAase by heating), 1 mg/ml, and kept for 12 hr at 45°. After cooling to room temperature, the DNA was precipitated by slowly pouring the mixture into two volumes of absolute ethanol. The precipitate was wound on a glass rod and dissolved in 0.14 M sodium chloride. The ethanol precipitation was repeated three times. DNA was determined according to Webb and Levy [6].

Residual RNA and oligonucleotides were separated from DNA by chromatography on hydroxyapatite [7]. Hydroxyapatite, Bio-Gel-HT from Calbiochem, in a 1.3 × 5 cm column, at 4°, was equilibrated with 200 ml, 1 mM potassium phosphate buffer, pH 6.8. After loading, the nucleic acids were eluted with a

linear gradient of potassium phosphate buffer, pH 6.8; the flow rate was 12 ml per hr.

For liquid scintillation counting, the mixture prepared as described by Butler [8] was used. The samples were counted in a Tracerlab liquid scintillation counter.

Chromatography of labelled nucleic acid bases was performed as follows: cells from a 24 hr culture in YPG containing 2-^{14}C-TMP, 0.5 Ci/ml, were washed, resuspended in 70% perchloric acid, and hydrolyzed for one hour at 100°. Following neutralisation with KOH and centrifugation, the pyrimidines were separated on chromatography paper, Schleicher and Schüll 2043 B by ascending chromatography with *n*-butanol-water, 86:14. The spots were visualized under UV light and their radioactivity determined by cutting the chromatogram in 1 cm strips and counting in Butler's scintillation mixture [8]

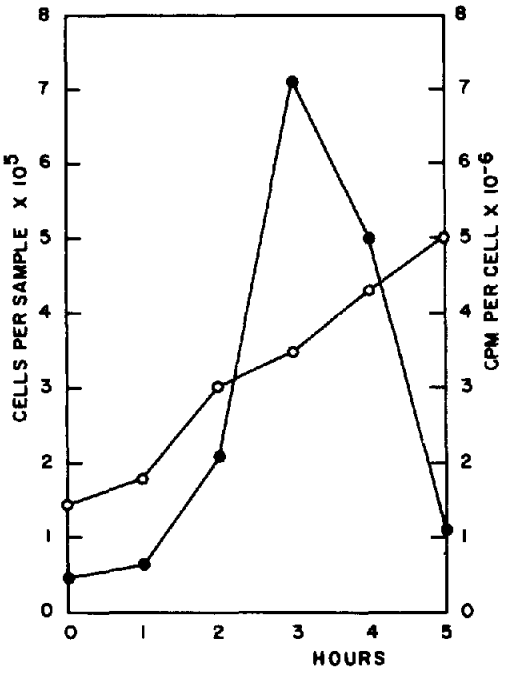

Fig. 1. Uptake of 2-^{14}C-TMP by cells of *S. cerevisiae*. Cells from a stationary culture were resuspended in fresh medium. 3 ml samples of the suspension were distributed in culture tubes and incubated at 30°. At the stated time, 0.3 μCi of 2-^{14}C-TMP was added to the tubes and the incubation was continued for 90 min. The cell titer (○——○) and the radioactivity in cpm per cell (●——●) was then determined.

3. Results and discussion

Fig. 1. shows that the rate of uptake of ^{14}C-TMP was dependent on the growth phase of the cells. Since the label was preferentially taken up by actively dividing cells, we concluded that exogenous TMP was incorporated into DNA. In order to confirm this, we tried to induce thymine dimers in labelled cells by UV irradiation. Radioactive dimers were thought to be formed only if the label was incorporated into DNA. As expected, the paper chromatogram of the perchloric acid extract of UV treated cells revealed thymine dimers (fig. 2). In addition, the chromatogram yielded information related to the specificity of incorporation of labelled TMP into the nucleic acids. Assuming a degradative pathway in yeast which converts thymine from TMP into uracil and cytosine, the incorporation of the label both in DNA and RNA is expected. Since we found neither radioactive uracil nor radioactive cytosine we concluded that exogenous

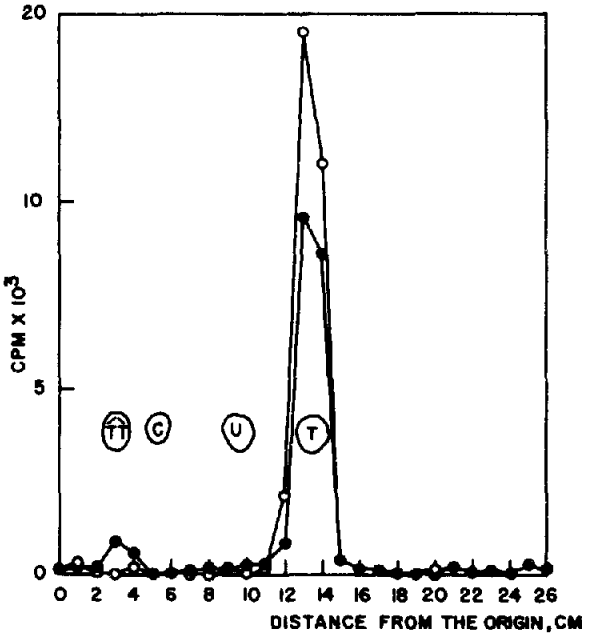

Fig. 2. Separation by paper chromatography of the pyrimidine bases and thymine dimers of UV irradiated (●——●) and unirradiated cells (○——○) of *S. cerevisiae*, labelled with 2-^{14}C-TMP. The cells were irradiated at room temperature for 30 min with a germicidal lamp (Sterisol, Hanau) at a distance of 5 cm. TT, thymine dimers; C, cytosine; U, uracil; T, thymine.

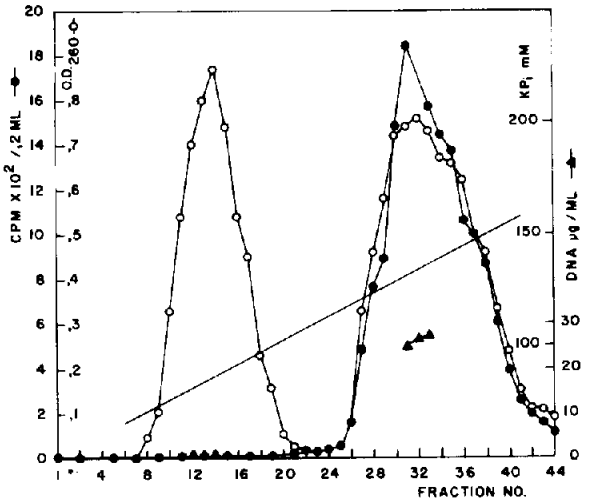

Fig. 3. Chromatography of nucleic acids of 2-^{14}C-TMP labelled cells of *S. cerevisiae* on hydroxyapatite. Elution was carried out with a linear gradient of potassium phosphate (KP_i), pH 6.8. 1 ml fractions were collected and 0.2 ml in 10 ml scintillation mixture [8] were used for liquid scintillation counting.

TMP was incorporated solely into DNA.

The strongest evidence for the specific labelling of DNA came from an experiment in which the nucleic acids from cells grown in 2-^{14}C-TMP containing medium were separated on hydroxyapatite. As shown in fig. 3, the label was confined to the second peak which contained the DNA.

In summary, we have shown that, in yeast, newly synthesized DNA can be labelled with 2-^{14}C-TMP. But we must add a note of precaution: for unexplicable reasons, not all commercial preparations of 2-^{14}C-TMP can be used for this purpose. We recommend testing of the specificity of incorporation for each batch of labelled TMP. In our hands, the products from the New England Nuclear Corporation gave very reproducible results.

Acknowledgements

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