EXPRESSION OF A YEAST EPISOME: RNA·DNA HYBRIDIZATION STUDIES

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1. Introduction

We have previously described a novel hereditary factor implicated in the drug resistance of yeast (*Saccharomyces cerevisiae*). The resistance was not determined by the mitochondrial DNA but was related to the presence of a circular DNA molecule of 2 μm circumference. In particular we observed a one to one correlation between the loss of oligomycin resistance conferred by the genetic factor Π carried by the mutant and the loss of the 2 μm circular DNA (Π-DNA) [1]. The presence in Π^+ strains or the absence in Π^- strains of closed circular DNA was checked by CsCl propidium diiodide gradients [2]. No band of closed circular DNA could be detected in the oligomycin sensitive Π^- strains. However, one could advance a hypothesis according to which these negative results would be due to the impossibility of extracting the circles from the Π^- strains either because they are destroyed or because they are very strongly bound to membranes for example.

Although the structure of this Π-DNA containing inverted repeats has been well established [3–5] very little is known about the in vivo transcription of this DNA.

The purpose of the present work was 2-fold: (1) To see whether a specific RNA complementary to the Π DNA could be found in yeast cells. (2) To see whether specific Π-RNA transcripts could detected in the Π^- strain in which no Π circular DNA could be extracted.

We performed RNA·DNA hybridization experiments with the RNA extracted from the oligomycin resistant strain (OLI^R) Π^+ and the oligomycin sensitive strain (OLI^S) Π^- and the Π-DNA, which simultaneously prove that the 2 μm circles are transcribed and that the absence of 2 μm circles in the strain Π^- does not result from the non-extractibility of these circles.

2. Materials and methods

2.1. *Yeast strains*

Total RNA was extracted from the oligomycin resistant clone DRI $9/T_3$ which carries the genetic determinant Π^+ and 2 μm circular DNA, and from the oligomycin sensitive clone DRI $9/T_4$ which does not carry the genetic determinant Π and is devoid of 2 μm circular DNA [1].

2.2. *Preparation of* Π *DNA*

The source of Π-DNA was λ phage no. 6 recombined in vitro with the Π-DNA extracted from the mutant OLI^R Π^+ DRI $9/T_3$ [6]. The methods of phage growth and DNA preparation have been previously described [6]. λ-DNA non-recombined in vitro with Π-DNA was used as non homologous DNA for preparing the blank filters.

2.3. *Preparation of* ^{32}P *RNA*

Homogeneously-labelled (steady state labelled) total RNA of aerobically grown cells was prepared as follows. The labelling was performed as described by Rubin [7] and the RNA specific activity was 2 to $4 \cdot 10^5$ cpm/μg. The cells were broken in a Vibrogen shaker and the RNA was extracted and purified using the method of Fraser [8]. It was further purified [9] to eliminate polyphosphate-like contaminants. Then it was treated with 10 μg/ml of

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pancreatic DNAase (free of RNAase) for 30 min. at 37°C. The DNAase was removed with phenol and after precipitation the purified RNA was dissolved in 0.1 SSC (standard saline citrate 0.15 M NaCl, 0.015 M Na citrate) at a concentration of 2 mg/ml.

2.4. *Hybridization*

The DNA in 0.1 SSC was denatured by heat and then immobilized on 25 mm nitrocellulose filters [10]. The RNA dissolved in 0.1 SSC was heat denatured (10 min in a boiling bath). The RNA·DNA hybridization experiments were then performed in 0.5 ml of 50% formamide, 2 SSC, 0.1% sodium dodecyl sulfate, 0.01 M TES (*N*-tris (hydroxymethyl) methyl-2 amino-ethane sulfonic acid (Calbiochem)). The hybridizations were done at 37°C for 60 h [11]. Each point is the mean value of three filters.

3. Results

Two types of experiments were performed. The first one was designed to test the difference between the two strains OLI^R Π^+ and OLI^S Π^- by removing all the Π-RNA transcripts from the total RNA (RNA constant-DNA increasing); the second one was designed to estimate the extent of hybridization of the Π circular DNA with the in vivo RNA transcripts (DNA constant-RNA increasing). The hybridization curves of the first experiment are shown in fig.1 and two different curves were obtained. When the total RNA extracted from the strain OLI^R Π^+ is used for hybridization a typical curve is obtained with a plateau corresponding to the removal of the RNA species homologous to Π-DNA. In contrast, when the total RNA extracted from the strain OLI^S Π^- was used no hybridization could be detected whatever the amount of Π-DNA on the filter. One should note that in this experiment the [^{32}P]RNA background was constant and did not vary with respect to the amount of Π-DNA on the filters. To check this result we repeated this experiment by using Π circular DNA extracted from the strain OLI^R Π^+ [1] instead of Π-DNA cloned on bacteriophage lambda. The same pattern of hybridization was obtained.

In the second type of experiment the amount of Π-DNA on the filters was kept constant and variable amounts of total RNA extracted from the strain

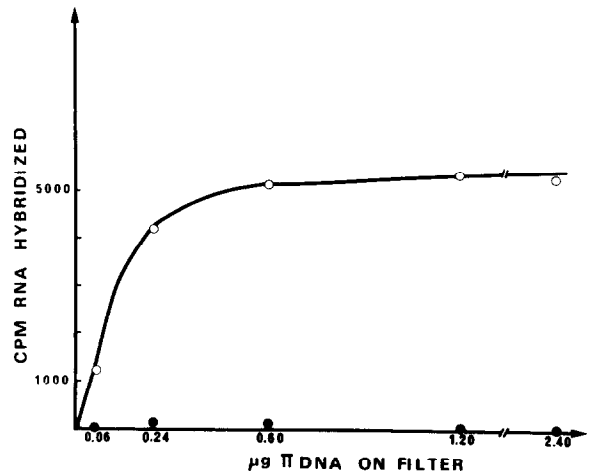

Fig.1. Exhaustion curves of total RNA hybridized with Π-DNA. The λ 6 DNA immobilized on the filters is expressed as Π-DNA equivalent. 40 μg of total RNA from the mutant OLI^R Π^+ (○——○), and from the revertant OLI^S Π^- (●——●), are dissolved in 0.5 ml of hybridization buffer. In these experiments the value of the blank was 2600 cpm for the total RNA OLI^R Π^+ and 2500 for the total RNA OLI^S Π^-. The specific activities of the RNAs were $2.8 \cdot 10^5$ cpm/μg.

OLI^R Π^+ were added to the hybridization buffer. The results are shown in fig.2. A typical hybridization curve was obtained and the maximum was

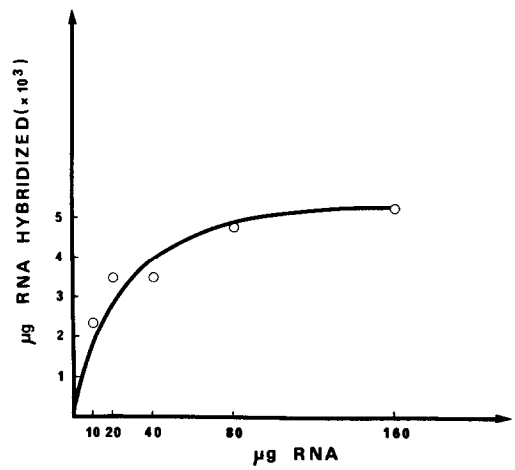

Fig.2. Saturation curve of total RNA OLI^R Π^+ hybridized with Π-DNA. The amount of λ 6 DNA immobilized on the filters corresponds to 0.1 μg of Π-DNA. The RNA was dissolved in 0.5 ml of hybridization buffer.

reached when 80 μg of RNA were added in the 0.5 ml of hybridization buffer. This maximum corresponds to 5×10^{-3} μg of RNA hybridized for 0.1 μg of DNA immobilized on the filters.

4. Discussion

The interest of the present work is 2-fold: (a) It shows that Π-DNA is transcribed in the strain OLI^R Π^+. (b) There are no Π-RNA transcripts in the strain OLI^S Π^- which has lost both oligomycin resistance and Π 2 μm circular DNA.

Two remarks concerning point (a) can be made:

(1) In addition to the above mentioned strains, we have also analyzed a wild type strain which contains 2 μm circles as do most of the yeast strains and is oligomycin sensitive. The same proportion of RNA transcripts homologous to 2 μm circles was found in this wild type strain which shows that the oligomycin resistance of the mutant is not due to a quantitative change in the transcription of the 2 μm circles. This is in agreement with our previous paper [3] which showed that the acquisition of drug resistance was not due to rearrangements or insertion of large fragments into Π circles.

(2) Taken at their face value the results shown in fig.2 indicate that 5% or 10% of the Π circles sequences were transcribed depending on whether transcription was symmetrical or asymmetrical. This low value could be due either to a limited transcription of the Π-DNA sequences or to a very low concentration of the homologous Π-RNA species which did not allow a real plateau to be reached. It is well known that transcription products may be in very low concentration (e.g.,) some mitochondrial RNA species [12]).

Concerning point (b) the present work confirms our previous conclusions on the absence of 2 μm circles in OLI^S Π^- strains [1]. The previous test was based on the presence or the absence of a heavy band of 2 μm closed circular DNA in a CsCl propidium diiodide gradient. One could imagine that the loss of closed circular DNA was due to a specific nicking or a specific lack of extractibility in the strain OLI^S Π^-. Present experiments demonstrate that the strain OLI^S Π^- differs from the strain OLI^R Π^+ in two respects since it has neither II circular DNA nor II-RNA transcripts.

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