# DIFFERENTIAL RNA SYNTHESIS IN THE MITOTIC CYCLE OF *PHYSARUM POLYCEPHALUM*

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Received 11 October 1973

## 1. Introduction

Many experiments indicate that the different classes of RNA molecules are made at unequal rates at the different times of the mitotic cycle of *Physarum polycephalum*. The incorporation of uridine shows two maxima per cell cycle, one of which is more sensitive to actinomycin than the other [1]. Both the base composition and nearest neighbor frequency of newly made RNA show consistent differences over the mitotic cycle [2, 3] pointing to more ribosomal RNA synthesis in the G-2 period and more non-ribosomal RNA synthesis in the S-period. Experiments with α-amanitin are consistent with this interpretation [4]. In order to detect differential transcription, pulse-labelled RNA has been examined by molecular hybridization [5]. It could be concluded that similar repeated sequences of DNA are transcribed at all times of the mitotic cycle, but no statement could be made regarding transcription of unique sequence DNA. To tackle this problem, further hybridization studies were carried out at high cot values and high salt concentrations [6]. Competition hybridization with RNA from different cell cycle times indicates differential transcription at specific stages of the mitotic cycle.

## 2. Materials and methods

### 2.1. *Preparation of [$^3$H]DNA*

Microplasmodia were labelled in shake culture for 24 hr with 5 μCi/ml of [$^3$H]thymidine. Nuclei were isolated according to Mohberg and Rusch [7] and incubated for 30 min at 37°C in SSG containing RNAase and amylase (50 μg/ml each). SDS was added to a final concentration of 2% and the lysate was then digested with 100 μg/ml of proteinase K by incubating for 30 min at 60°C and then for 2 hr at 37°C. If the solution did not become clear the proteinase treatment was continued with more enzyme. After deproteinisation with chloroform–isoamylalcohol, DNA was precipitated twice with ethanol and spooled. It was then dissolved in 1/10 SSC and banded in a CsCl gradient for 36 hr at 42 000 rev/min in a fixed angle Ti 50 rotor.

### 2.2. *Preparation of [$^{14}$C]RNA*

Surface cultures whose last mitosis had been determined were labelled for 1.5 hr with 5 μCi/ml of [$^{14}$C]uridine. The plasmodia were extracted by a modification of the procedure of Holt [8]. They were homogenized for 30 sec in an Omnimix in a solution containing 5% TNS, 10 mM EDTA, 10 mM Tris, pH 7.2, 1% NaCl, 3% Bayocovin. For 3 standard surface cultures 10 ml were used. For deproteinization 10 ml phenol–cresol–hydroxyquinoline (50 ml/7.5 ml/



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50 mg) were added and the mixtures shaken for 30 min at room temperature. After centrifugation for 5 min at 5000 rev/min in a Sorvall HS 4 rotor the interphase was extracted twice more at 60°C. The RNA was precipitated with ethanol from the aqueous phase, dissolved in 10 mM acetate buffer pH 5.3, run in the same buffer through a column of Sephadex G 25 and precipitated once more with ethanol.

2.3. *Hybridization procedure*

Labelled DNA in 1/10 SSC was heated for 10 min to 100°C, rapidly chilled and trapped on a large Millipore filter (11 cm diam.) which had been presoaked in 4 × SSC [9]. After filtration by gravity, the Millipore membrane was washed on both sides with 4 × SSC, dried at room temperature and baked for 4 hr at 80°C in a vacuum. The filter was treated after Denhard [10] and disks of 5 mm diam. were punched out. The DNA content was about 1 μg per filter.

Blank filters were subjected to the same treatment, but in the absence of DNA. In small glass vials 2 blank filters and 2 DNA filters were incubated for 8 days at 38°C in 100 μl of 50% formamide, 1 M sodium perchlorate, 1 × SSC, 20 mM phosphate buffer pH 7.2 and the desired amounts of RNA. After incubation the filters were washed in the same solution but without formamide and treated for 1 hr at 37°C with 10 μg/ml RNAase. The filters were washed again, dried and counted.

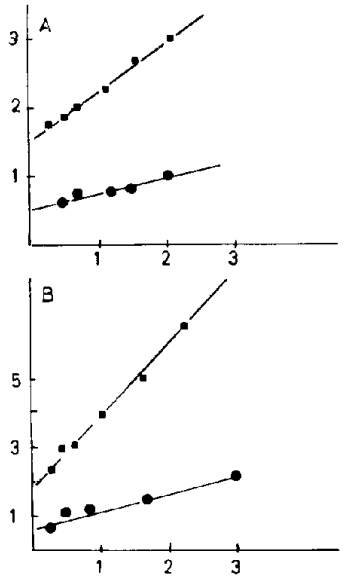


Fig. 1. (A) Kinetics of hybridization. Abscissa: 1% Hybrid × 10; Ordinate: 1/hr × $10^2$. (▪—▪—▪) RNA, isolated 0.5 hr before mitosis. (•—•—•) RNA, isolated 2 hr after mitosis. (B) Dependence of hybridization on RNA concentration. Abscissa: 1% Hybrid × 10; Ordinate 1 μg RNA $ml^{-1} \cdot 10^2$. (▪—▪—▪) RNA, isolated 0.5 hr before mitosis. (•—•—•) RNA, isolated 2 hr after mitosis.

## 3. Results and discussion

3.1. *Kinetics and saturation of hybrid formation*

RNA labelled in the S-Phase and the G-2 phase was hybridized for an increasing length of time to DNA. Fig. 1A shows the results of such an experiment in the form of a double reciprocal plot. The time required to reach half saturation is in good agreement with the value of 6 days expected for 500 μg/ml RNA from the kinetic complexity of *Physarum* DNA, which has been estimated to correspond to about 130 *E. coli* genomes. The saturation values obtained show that more DNA sequences are transcribed in the S-phase than in the G-2 phase, thus demonstrating differential transcription. For the S-phase the saturation level was 14%, for G-2, 5%. Varying the RNA:DNA ratio at a fixed hybridization time of 192 hr gave the result shown in fig. 1B. For G-2 phase RNA saturation was reached at a ratio of 200:1; for S-phase RNA at a ratio of more than 500:1. From this we conclude that the S-phase RNA contains sequences not present in the G-2 RNA.

3.2. *Competition hybridization*

In these experiments DNA was pre-hybridized with increasing amounts of unlabelled RNA from the early S-phase or the late G-2 phase. In a second step the filters were exposed to a large excess of a labelled RNA from the two-cell cycle stages. Fig. 2 shows double reciprocal plots of such an experiment. Ii is seen that homologous RNA is a better competitor than heterologous RNA. These observations are in agreement with our previous conclusions that there is a differential transcription in the mitotic cycle. The conclusion has to pertain to unique sequence DNA, since hybridization was done for very long periods and with a high RNA:DNA ratio.

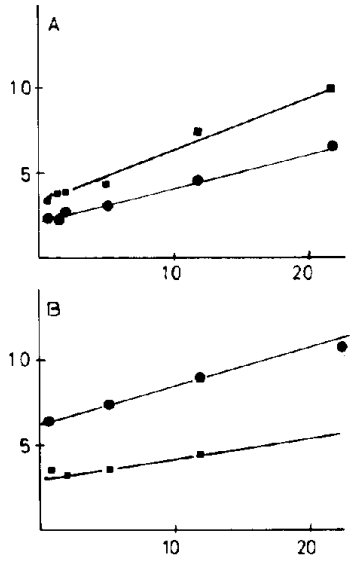


Fig. 2. Competition hybridization of *Physarum* RNA, isolated at different times of the cell cycle. Abscissa: 100/100 - % Hybrid, Ordinate: 1 μg RNA $ml^{-1} \cdot 10^2$. Each assay contained 1 μg DNA fixed on a filter and different amounts of unlabelled RNA. After 8 days hybridization time, the filters were washed and incubated with 500 μg pulse-labelled, homologous or heterologous RNA for another 8 days. (A) RNA pulse-labelled 2 hr before mitosis for 90 min. (▪—▪—▪) Competition against heterologous RNA, isolated 2 hr after mitosis. (•—•—•) Competition against homologous RNA, isolated 30 min before mitosis. (B) RNA pulse-labelled 0.5 hr after mitosis for 90 min. (▪—▪—▪) Competition against homologous RNA, isolated 2 hr after mitosis. (•—•—•) Competition against heterologous RNA, isolated 0.5 hr before mitosis.

## Acknowledgements

The hybridization conditions were elaborated with the help of Dr. H.W. Sauer, Heidelberg. We wish to thank Kathryn Behrens for excellent technical assistance. These investigations were supported by a Ausbildungsstipendium of the Deutsche Forschungsgemeinschaft and grant No. 3.750.72 of the Swiss National Science Foundation.

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