TRANSCRIPTION OF RIBOSOMAL RNA IN THE LIFE CYCLE OF PHYSARUM MAY BE REGULATED BY A SPECIFIC NUCLEOLAR INITIATION INHIBITOR

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SUMMARY
Physarum nucleoli contain an inhibitor of in vitro transcription with homologous RNA polymerase A. A strict negative correlation has been established of RNA polymerase A activity and amount of inhibitor during differentiation of Physarum. Location and concentration of the inhibitor as well as selective, yet reversible, binding to and inactivation of RNA polymerase A and in vitro reactivation of enzyme A preparation obtained during differentiation - but not during growth - suggest that the inhibitor might act in vivo to restrict rRNA transcription.

INTRODUCTION

Cells react to an adverse environment in many ways at favour survival. Although the exact mechanism of pleiotypic control (1) is not known, a low activity of RNA polymerase A (EC 2.7.7.6) and ribosomal RNA transcription has been observed in eukaryotes as in bacteria, where guanosine tetraphosphate might be involved in the stringent control mechanism (2-5). A high polyphosphate level at times of low nucleic acid synthesis has been known for some time (6). However, a direct involvement of such molecules in the regulation of transcription of ribosomal RNA has not been shown. We wish to present evidence that a high molecular weight organic polyphosphate (7,8) plays a specific regulatory role during the encystment of Physarum, a differentiation process which is accompanied by a significant reduction of ribosomal RNA

(9-12) and a drastic decrease of RNA polymerase A activity (13).

MATERIAL AND METHODS

Physarum was grown as a suspension of microplasmodia or individual macroplasmodia and spherulation or sporulation was induced according to standard procedures (14). RNA polymerases A and B were isolated and assayed on denatured calf thymus DNA as reported previously (15). The inhibitor was detected as described previously (8), purified by velocity gradient centrifugation, ion exchange chromatography and gelfiltration and tested for inhibition of a complete RNA polymerase assay. The inhibitor was quantitated as dry weight or acid labile phosphate per 10^6 nuclei. Transcription of native calf thymus DNA with RNA polymerase A was reduced by 10 % or 90 % at 0.5 µM and 5 µM phosphate of the inhibitor respectively. In some experiments labeled inhibitor was isolated after incubating microplasmodia in 100 µCi ^{32}P orthophosphate/ml medium for 20 hrs during the transition from exponential to stationary growth phase.

RESULTS AND DISCUSSION

In a series of experiments levels of RNA polymerases and of a nuclear transcription inhibitor have been determined in aliquots from the same culture of Physarum over the period of spherulation (Table 1). We observe characteristic fluctuations in the specific activity of enzyme A, whereas RNA polymerase B remains rather stable, confirming an independent control mechanism for these two nuclear RNA polymerases (13). The amount of the isolated transcription inhibitor, based on its weight or acid labile phosphate or on the degree of inhibition of a standard RNA polymerase assay, increases as the RNA polymerase A activity level decreases. This negative correlation is also seen at later stages of spherulation and germination when enzyme A activity rises again. Similar observations are made in aged macroplasmodia (Table 1) which have become competent for induction of sporulation (14). However, no alterations in RNA polymerase activity or inhibitor level are seen during the sporulation process (Table 1).

TABLE 1. Correlation of RNA polymerase activity and an endogenous transcription inhibitor during differentiation of Physarum

Microplasmodia Hours in salt medium	RNA polymerase activity* Enz.B	Enz. A	Amount of inhibitor**	Relative Inhibition***
0	100	100	0.08	10
4	100	50	0.46	62
8	90	10	1.12	96
12	95	30	0.74	81
16	100	65	0.43	63
20	100	70	0.40	52
Spherules	100	100	0	0
Germination	100	100	0.06	10
Macroplasmodia Days in growth medium				
1	100	100	0.09	15
2	100	80	0.21	28
4	90	20	1.04	91
6	75	25	0.98	88
8	80	40	0.62	73
8 (Sporulation)	80	40	0.64	75

* Approx. equal specific activity of both RNA polymerases (2 u/mg protein, 1 u = 1 mMole UMP incorporation/10 min, 25°) is detected during logarithmic growth. These control values are set 100 %. The specific activities of the enzymes during differentiation are expressed relative to the controls, triplicate enzyme determinations are within ± 5 %.

** Dry weight /ug/culture containing 10^6 nuclei, ± 10 %.

*** Inhibition of a standard RNA polymerase assay (each with the amount of purified enzyme A, extracted from an exponentially growing culture containing 10^6 nuclei) by the inhibitor obtained from a culture containing 10^6 nuclei. Results from triplicate experiments are within ± 10 %.

Since the differential changes of RNA polymerase activity shown in Table 1 cannot be explained by selective turn-over (13), we postulate that RNA polymerase A activity might be regulated in vivo by the endogenous inhibitor on the basis of the following experiments:

The inhibitor has been prepared from isolated nuclei or nucleoli yielding the same variations in content and inhibition as those from total plasmodia presented in Table 1. Furthermore, after an incubation of Physarum plasmodia during differentiation in ^{32}P orthophosphate, labeled inhibitor has been found predominantly in nucleoli. Mixing experiments of purified labeled inhibitor with nucleoli have ruled out unspecific adsorption during the cell fractionation procedures. These experiments indicate that the inhibitor is located in the nucleolus, the site of RNA polymerase A.

The amount of inhibitor, isolated at 8 hrs of starvation in salt medium or after 4 days of growth (approximately 1 µg/10^6 nuclei, Table 1), is sufficient to inactivate 90 % of theRNA polymerase A molecules which can be obtained from an exponentially growing culture with 10^6 nuclei, whereas only partial inhibition (10 %) can be observed by the 0.08 µg inhibitor/10^6 nuclei, extracted from exponentially growing cultures. According to these results there is enough inhibitor present in vivo to explain the decrease of RNA polymerase A activity during spherulation. The purified inhibitor inactivates RNA polymerase A activity by 50 % at a concentration of 2 µM acid labile phosphate. At this concentration RNA polymerase B from Physarum, RNA polymerase A from mouse L cells and RNA polymerase from E.coli are inhibited less

TABLE 2. Binding of ^{32}P labeled inhibitor to RNA polymerases

Treatment	Inhibitor-enzyme-complex* with		
	RNA pol. A	RNA pol. B	E.coli RNA pol.
none	22	21	20
0.3 M NH_4Cl	19	20	18
0.5 M NH_4Cl	18	0	0
1 M NH_4Cl	0	0	0

*) Percent of inhibitor (100 % = 65000 cpm) recovered at the position of the RNA polymerase in the glycerol gradient.
^{32}P labeled inhibitor was incubated with the enzyme (0.1 unit of each highly purified enzyme, 400 units/mg protein) at 0^{o} for 15 min (resulting in complete inhibition) and then centrifuged on linear 10-30 % glycerol gradients for 12 hrs at 50000 rpm. The factor sedimented at 4 S, the enzyme at 15 S.

than 10 % of control assays without inhibitor (16, and unpublished results). Furthermore, the labeled inhibitor (4S) binds to RNA polymerase (15 S) in glycerol gradients (Table 2). This complex is resistant to salt up to 0.3 M for any of the RNA polymerases tested. However, only the complex with the homologous enzyme A is stable at 0.5 M NH_4Cl which is consistent with a selective inhibition of this enzyme. At still higher ionic strength the ^{32}P labeled inhibitor is lost even from RNA polymerase A. This is paralleled by a reactivation of the RNA polymerase A at 0.8 M NH_4Cl (Table 3). The same effect is seen by repeated ammoniumsulfate precipitations (Table 3).

Interestingly, our experiments suggest that RNA polymerase A

TABLE 3. Reactivation of RNA polymerase A from Physarum

Treatment	Source of RNA pol. A*		
	Growth		Starvation
	Control	Control + Inhibitor	
none	100**	10**	10**
high salt	100	90	55
ammonium sulfate	100	90	75

*) RNA polymerase A was determined from aliquots of cultures during exponential growth or 8 hours of starvation in salt medium either after chromatographic separation of the two enzymes or in the homogenate as amatoxin (50 µg/ml) resistant enzyme activity.

**) Percent UMP incorporation by RNA polymerase A preparations obtained from logarithmic growth cultures (100 % = 55000 cpm/10 min at 30°), after addition of the inhibitor (5 µM phosphate) or from spherulating cultures.

) Each of the three enzyme preparations after centrifugation in the presence of 0.8 M NH_4Cl on 10-30 % linear glycerol gradients.

) Enzyme activity of the homogenate or after chromatographic separation of the enzymes after 4 cycles of ammonium sulfate precipitation.

molecules from Physarum are complexed with the inhibitor *in vivo*. The same treatments which reactivate RNA polymerase A after its inactivation *in vitro* by the addition of purified inhibitor, also increase (Table 3) the low RNA polymerase A activity of enzyme preparations obtained from spherulating cultures at the stage of minimum apparent specific activity of enzyme A (8 hrs in salt medium; see Table 1).

Our observations on the mechanism of inhibition by the endogenous inhibitor are consistant with the following model of transcription control of ribosomal cistrons in Physarum: In response to conditions of starvation a specific inhibitor of

RNA polymerase A is synthesized, or accumulated, in nucleoli. This inhibitor binds to - and thereby inactivates - free RNA polymerase A molecules which are abundant in Physarum (13) until the inhibitor-enzyme complex is dissolved. It is worth mentioning that labeled factor disappears *in vivo* after refeeding (17) and that homogenates of isolated nuclei from growing Physarum degrade labeled factor. Our results show that the pleiotypic control mechanism in Physarum might involve a transient increase in the level of an rRNA transcription inhibitor.

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