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APPEARANCE OF POLYADENYLATED RNA SPECIES DURING SPORULATION IN *BACILLUS POLYMYXA*

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SUMMARY

Two main features of altered transcriptional programming during sporulation are reported; 1. The occurrence of the polyA RNA species exclusively during sporulation. 2. The localisation in the membranes of regions of DNA that may be enriched in spore genes.

INTRODUCTION

Sporulation specific transcriptional activity has been identified either by way of appearance of altered hybridization capacity of mRNAs appearing during sporulation (1,2,3) or by the possible alteration of the RNA polymerase by the appearance of new polypeptides associated with RPase during sporulation (4,5). We had reported the appearance of membrane DNA (mDNA) associated transcriptional activity at the onset of sporulation of *B.polymyxa* and had suggested that this phenomenon was a result of the increased amenability of regions of DNA for the transcriptional machinery during sporulation (6). This transcriptional activity was present

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The initial hybridization studies reported here were carried out in the laboratory of Dr R.H.Doi at University of California, Davis during the tenureship of Fulbright Senior Fellowship to KJ.

Abbreviations: RPase - RNA polymerase; mDNA - membrane DNA; mRNA - messenger RNA ; SDS - sodium dodecyl sulphate

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as a tightly bound complex with mDNA and was highly resistant to rifamycin, while the soluble transcriptional activity was sensitive to rifamycin (6).

These results indicated the special involvement of regions of DNA associated with membrane during sporulation. The present studies are concerned with the differences in the populations of mRNAs during the growth and sporulation stages.

MATERIALS AND METHODS

Microorganisms: The organism used in the course of these studies was Bacillus polymyxa obtained from Pfizer and Co. USA. The Rif^r, SpoO mutant was isolated as described earlier (6).

Media: The rich medium of Schaeffer (7) was used.

DNA extraction: DNA was extracted from whole cells and the membrane fractions after phenol deproteinization. Membrane fractions were prepared as described earlier (6).

Extraction of labelled RNA: For hybridization studies, the RNA was labelled by pulsing the growing cultures of B.polymyxa at the vegetative phase with 3 - 5 mCi of ^{3}H-uridine for 3 min and at the T_2 phase with 15 mCi for 15 min. For the extraction of polyA RNA species, the cells were labelled by growing in the presence of 100 uCi of ^{3}H-adenosine in 50 ml of medium and harvesting them at the vegetative phase and the T_2 phase (2 hrs after the end of growth). The cells were resuspended in 10 ml of RNA extraction buffer containing Tris-HCl, 10 mM, pH 7.4, 10 mM magnesium chloride and 1 unit/ml of heparin. Lysozyme (1mg/ml) and DNase (30 ug/ml) were added to the mixture and incubated at 37°C for 30 min. SDS at a final concentration of 0.2% was added and the lysed cells were immediately deproteinized with an equal volume of phenol:chloroform:isoamyl alcohol (50:48:2) mixture containing 0.1% 8'hydroxyquinoline. The deproteinised aqueous phase was precipitated with 95% ethanol and left overnight at -10°C, centrifuged and dissolved in the same buffer.

DNA:RNA hybridization: This was carried out according to the method of Nygaard and Hall (8). The hybridization was carried out under conditions of RNA excess. The reaction mixture, in addition contained 50% formamide (v/v) and citrate buffer (0.15 M sodium chloride and 0.015 M sodium citrate. pH 7.0) at a final concentration of 6X, and was

incubated for 24 hrs at 37°C. At the end of the incubation period the mixture was cooled in ice and filtered on nitrocellulose filters, presoaked in 2X citrate buffer. The filters were washed with 2X citrate buffer (75 ml) and incubated with stirring in the presence of 20 ug/ml pancreatic RNase in 3 ml of the 2X citrate buffer for 30 min at 30°C. The filters were washed with 50 ml of 2X citrate buffer on both the sides, dried and counted in a liquid scintillation counter. In the control assays, DNA was omitted from the reaction mixtures.

Competition: The labelled DNA:RNA hybrids were chased by an equal amount of cold RNA.

PolyU filter binding assay: This was carried out by the method of Sheldon (9). RNA was dissolved in 10 ml of the binding buffer (0.05 M Tris-HCl, 0.2 M sodium chloride, 0.01 M EDTA) and was bound to the polyU filters. The filters were washed extensively with 2.5% cold trichloroacetic acid and ethanol.

Oligo dT cellulose chromatography: To one gram of oligo dT cellulose (generous gift of F.Wilt, University of California, Berkeley, USA) about 100 O.D units of RNA was loaded. The polyA containing RNA was bound to the column with a high salt buffer (10 mM Tris-HCl, pH 7.4, containing 0.5 M sodium chloride, 0.5% SDS and 1 unit/ml of heparin) and for subsequent elution Tris-HCl buffer containing 0.5% SDS and 1 unit/ml of heparin was used (10).

Fractionation of total RNA on sucrose density gradient:

A continuous sucrose density gradient of 5 - 15% (w/v) in 0.05 M sodium chloride and 0.01 M sodium acetate, pH 5.0 was made. ^{3}H-adenosine labelled samples of RNA (0.2 ml) were loaded on to the gradient and centrifuged in the swing out rotor in a Janetzki VAC 601 ultracentrifuge for 18 hrs at 90,000 g.

RESULTS AND DISCUSSION

Hybridization studies with pulse-labelled mRNA populations with the total DNA and the DNA associated with membrane fractions indicated that messengers at T_2 stage of sporulation hybridised preferentially with mDNA (Fig.1a). In contrast, the vegetative mRNA populations hybridised poorly with mDNA (Fig.1b). That there were increased homology for T_2 mRNA in the mDNA was evident by the higher rate and extent of hybridization of T_2 messengers with

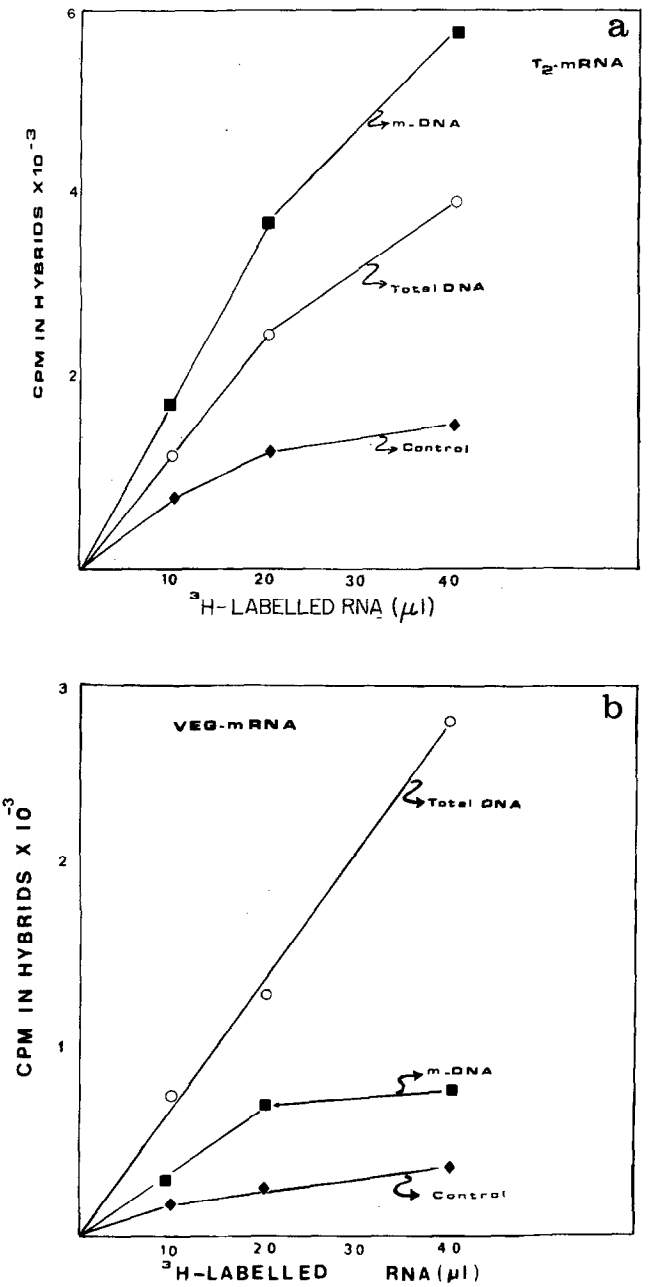

Fig.1

Kinetics of hybridization of mRNA species with total and mDNA: Different quantities of Pulse labelled mRNA isolated from the cells at the vegetative phase and at the T_2 phase were hybridized to mDNA and total DNA(1^2ug/assay). In the control assay DNA was omitted from the reaction mixture. a - T_2; b - vegetative.

Table I

Specificity of hybridization of T_2 messengers with the mDNA

RNA	CPM of RNA hybridized with [a]	
	Total DNA	mDNA
^3H vegetative RNA	2200	ND [b]
^3H vegetative RNA + unlabelled vegetative RNA	ND	ND
^3H vegetative RNA + unlabelled T_2 RNA	400	
^3H T_2 RNA	2970	3560
^3H T_2 RNA + unlabelled vegetative RNA	700	3000
^3H T_2 RNA + unlabelled T_2 RNA	500	200

a - ^3H-adenosine labelled RNA (15 ug/assay) from vegetative and the T_2 stages was hybridized to 5 ug of total mDNA. Cold RNAs (15 ug/assay) were added at the beginning of the chase experiment. The blank values were subtracted in each case.

b - Not detectable

mDNA as compared to total cellular DNA. This specificity of hybridization was further confirmed by the chase experiments in which the mDNA-T_2 hybrids could not be competed by the vegetative mRNA (Table 1). However, there occured considerable hybridization of T_2-mRNAs with total DNA, most of which could be chased out by vegetative RNA, thus confirming the earlier reports of extensive transcription of vegetative genes during the sporulating stages (1,2).

In most of our hybridization experiments with T_2 messengers we had observed high blank values (Fig.1a and 1b). It is well known that RNA populations with stretches of polyA bind to the nitrocellulose filters at higher salt concentrations (11). Hence, we decided to look into the

Table II

Occurrence of the polyA stretches in the RNA populations of the vegetative and the T_2 stages of growth in *B.polymyxa*

Strain	Stage	Percentage of total RNA bound to	
		PolyU filters	Oligo dT columns
Wild type	Vegetative	1.2	4.0
	T_2	15.0	12.0
RS 01	Vegetative	1.2	Nil
(Rif^r, SpoO)	Stationary	1.8	Nil

Total RNA isolated from cells labelled with ^{3}H-adenosine were examined for the extent of binding to polyU filters and their retention on oligo dT columns. The values are expressed as the percentage of the total TCA-precipitable cpm of the input RNA.

possibility that the RNA populations may be endowed with a polyA tail. We had labelled all the RNA species at vegetative and T_2 stages by growing the cells in the presence of ^{3}H-adenosine. The presence and extent of RNA with polyA tract was analysed by polyU filter binding assays and fractionation of the total RNA on oligo dT cellulose column. The data presented in Table 2 clearly indicated that T_2 RNA populations alone were distinguished by the presence of polyA. That this was a spore-specific manifestation was revealed by the inability of RNA populations isolated from a Rif^r, SpoO mutant to either bind to polyU filters or oligo dT cellulose. Further confirmation of the presence of polyA tract in T_2 RNAs came from the demonstration of its sensitivity to T_2 RNAse.

In order to understand the nature of the species of RNA that are polyadenylated, we had separated the ^{3}H-adenosine

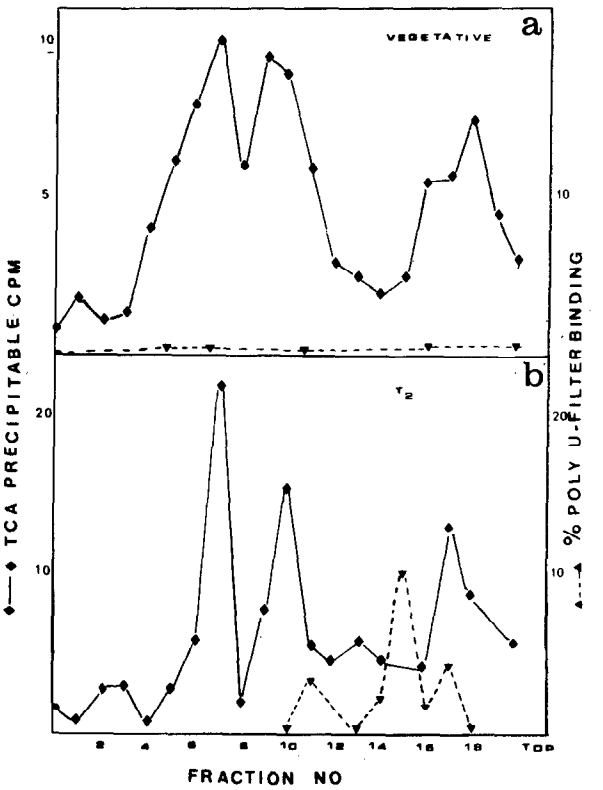

Fig.2

Density gradient profiles of ^3H adenosine labelled total RNA from vegetative&T_2 stages: Samples of labelled RNA (0.2 ml) were loaded on a 5 ml 5-15% (w/v) sucrose density gradient and centrifuged for 18 hrs at 90,000 g. The individual fractions were monitored for their total acid precipitable CPM (♦— ♦) and their polyU filter binding capacity (▼-- ▼). a - vegetative; b - T_2

labelled RNA species from vegetative and sporulating stages in a sucrose density gradient. Individual fractions were checked for their acid-precipitable counts and their polyU filter binding capacity. It is evident that the polyA tracts are present in 5-9S RNA species only at the sporulating stage (Figs. 2a and 2b).

The results of these investigations have brought forth yet another interesting feature of specialised transcriptional

programming during sporulation. We report here the occurrence of polyadenylated RNA as a specific feature of sporulating cells. The presence of polyA RNA in procaryotes has been reported (12,13). The role of polyA stretches in eucaryotic RNA has been speculated to be that of stabilising the messenger RNAs enroute their passage from the nucleus to the cytoplasm (14). Whether or not a similar role can be attributed for the polyA RNA species in sporulation is unclear at the moment.

In view of the enriched homology of mDNA regions with T_2 messengers and the ease of separation of polyA RNA from the total pool of RNA, we now have a powerful tool of localising the spore-specific regions in the cellular DNA as well as of studying the turnover of the polyA RNA species during sporulation.

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Note added in proof: The presence of polyA RNA in *B.brevis* has been reported recently (Sarkar,N., Langley,D and Paulus,H. Biochemistry. 17, 3468 (1978)

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