RNA METABOLISM DURING DIFFERENTIATION IN THE CELLULAR SLIME MOLD

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Introduction

Biochemical investigations of differentiation are often complicated by the occurrence of growth in the differentiating system. The cellular slime mold, Dictyostelium discoideum, differentiates without concomitant growth and thus affords an opportunity to study processes such as the RNA metabolism of differentiation more specifically. The purpose of this paper is to examine the rate of RNA turnover as a function of developmental stage; in the accompanying paper, Actinomycin D is used to investigate the possible interdependence of RNA turnover and differentiation.

Materials and Methods

D. discoideum, strain NC-4, was grown in the presence of E. coli, harvested, and then allowed to differentiate on non-nutrient agar (Liddel and Wright, 1961). Cells (approximately 0.3 ml packed cell volume) to be labeled with ^{14}C-uracil (obtained from Nuclear-Chicago Corp.) were rinsed off the agar in 3 ml Bonner's "standard solution" (buffered to pH 7.2 with 10^{-3} M Tris) and incubated at 23^{o} with gentle swirling in 50 ml Erlenmeyer flasks in the presence of ^{14}C-uracil (0.037 μmoles). In a separate experiment it was shown that this level of uracil did not affect either developmental rate or the composition of the acid soluble nucleotide pool. The cell volume and pool composition did not vary significantly during the periods of incubation employed ($<$ 30 min). Following incubation, the suspension was made 0.35 M in $HClO_4$, extracted for 30 min in the cold and the precipitate rinsed with an equal volume of 0.35 M

$HClO_4$. The combined supernatants were neutralized with KOH and comprised Fraction I. The precipitate was washed with an equal volume of 95% ethanol, taken up in 1 ml of 0.3 M potassium hydroxide and incubated for 18 hr at 37°. The hydrolysate was then acidified by the addition of 0.1 ml of 3.5 M $HClO_4$ and the resulting precipitate rinsed with 1 ml of 0.35 M $HClO_4$. The combined supernatants were neutralized with KOH and this fraction (Fraction II) was then analyzed by ion-exchange thin-layer chromatography (TLC) (Randerath and Randerath, 1965). The UTP of Fraction I was separated from the other nucleotides by 2-dimensional TLC (Randerath and Randerath, 1964) following purification of the fraction on acid-washed charcoal (Pannbacker, 1966). Nucleotides were quantitatively eluted from the chromatograms with 0.7 M $MgCl_2$ (0.02 M in pH 7.5 Tris buffer). The absorbance of the eluates at 260 mμ (A_{260}) was determined, and the radioactivity measured in a Packard Tri-Carb liquid scintillation spectrometer Model 314-DC.

Results and Discussion

Quantitative TLC of Fraction II showed that all of the A_{260} of this fraction could be accounted for by the sum of the absorbancies of the spots corresponding to the 2'-3'-monophosphates of uridine, adenosine, guanosine, and cytidine. The identity of these spots was confirmed by comparison of the R_P's and of the spectra of the eluates to those of authentic nucleotides. Extraction of the RNA of a ^{14}C-labeled acid-insoluble pellet with hot 10% NaCl (Hurlbert and Potter, 1952), followed by alkaline hydrolysis, yielded 2'3'-UMP of the same specific radioactivity as that of the directly hydrolyzed pellet.

The amount of RNA/mg dry weight was determined by relating the A_{260} of Fraction II from a known amount of cells to the average extinction coefficient of the mononucleotide mixture. The level calculated (roughly 100 μg/mg dry weight) agrees with that obtained by White and Sussman (1961) by another method. We were also able to show that RNA level/packed cell volume remains constant during development.

RNA Base Composition

The base composition of the bulk RNA was also found to remain essentially constant throughout differentiation. The average base composition determined in a typical experiment is shown in Table IA, along with the base composition of RNA from the mature spores. Table IB gives the average values obtained from a number of experiments as well as the range in reported values for 26 other species of fungi (Storck, 1965). The comparisons illustrate that the RNA of the slime mold has an unusually low G + C content. This is of interest in view of the extremely low G + C content of its DNA (Schildkraut et al., 1962).

TABLE I

Base Composition of Bulk RNA

	Mole %				
RNA source	G	U	A	C	G + C
A.					
Amoeba to culmination	22.0 ± 1.9*	33.6 ± 3.2	24.1 ± 2.3	20.4 ± 4.7	42.4
Mature spores	25.6	29.0	28.5	16.7	42.3
B.					
Slime mold					
26 expts. at all stages	22.1 ± 5.8	30.5 ± 6.0	28.8 ± 4.8	18.5 ± 4.5	40.6
Other species of fungi	23.4 - 34.6	19.4 - 27.6	20.0 - 30.9	19.2 - 25.9	44.1 - 60.5

*The interval indicates one standard deviation of the mean.

RNA Turnover

The rate of turnover of RNA is here defined as the percentage of the uracil in the RNA replaced in one hour by uracil of the specific radioactivity of intracellular UTP: RNA turnover (%/hr) = $\left(\frac{\Delta \text{cpm}/\mu\text{mole 2'3'-UMP/min}}{\text{cpm}/\mu\text{mole UTP}}\right)(60)(100)$.

The importance of basing the rate on the specific radioactivity of the immediate precursor is emphasized in Figs. 1 and 2. In Fig. 1 the rate of appearance of radioactivity in the uracil of RNA is compared under identical

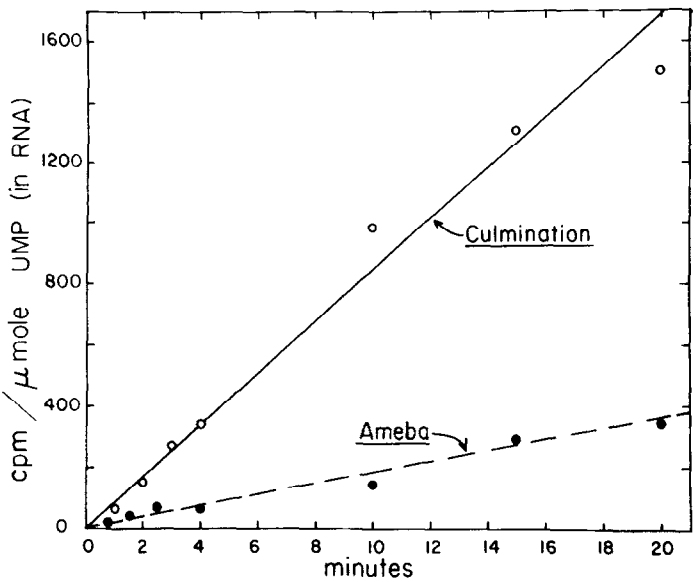

Fig. 1. The rate of appearance of radioactivity in UMP of RNA hydrolysates during incubation with ^{14}C-uracil, measured at the amoeba and culmination stages of development.

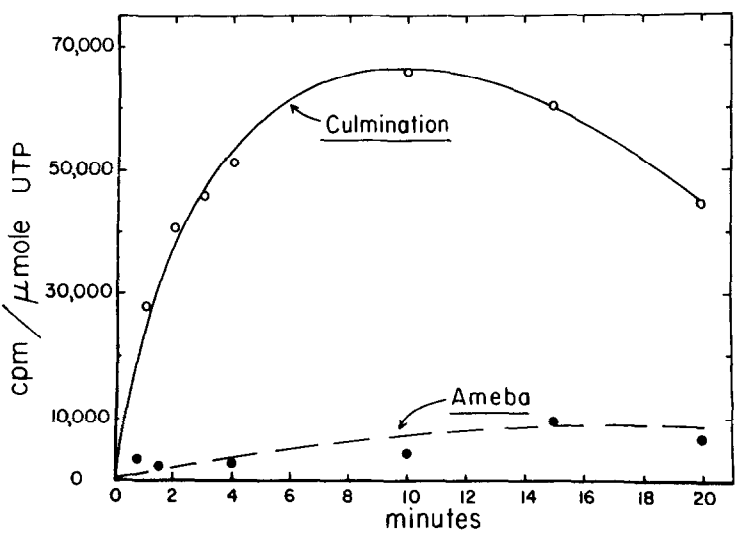

Fig. 2. The specific radioactivity of intracellular UTP during the experiment shown in Fig. 1.

incubation conditions at two stages of development. It is apparent that radioactive uracil is incorporated more rapidly at culmination, suggesting a higher rate of RNA synthesis at that stage than at the amoebae stage. However, when the rates of RNA synthesis were determined based on the specific radioactivity of the UTP pool during these incubations (Fig. 2), it was found that the cells at the culmination stage were actually synthesizing RNA at only half the rate of the amoebae. Thus comparison of rates of RNA turnover based simply on uptake of radioactive uracil is seen to give misleading results. Table II summarizes the results of several experiments in which the rate of turnover at various stages was investigated.

TABLE II

Turnover of RNA Uracil (%/hour)

Experiment No.	Stage			
	Amoeba	Agg.	Slug-Preculm.	Culm.
1			25.4, 28.5	11.0
2		9.6	13.4	
3			15.4	
4	25.4	8.2		9.0
5	7.1	6.3		
6	6.5	11.4		
7		6.7	13.4	

No consistent change in rate was observed during development. These results are comparable to and consistent with data on the rate of protein turnover during differentiation. With this product, also, it was found essential to calculate the rate based on the specific radioactivity of an intracellular precursor, methionine (Wright and Andersen, 1960a, b).

The lack of change in RNA level, base composition, and rate of turn-

over suggests that no major quantitative changes in the RNA metabolism of this organism occur during multicellular development. A further attempt to evaluate the significance of RNA metabolism for this developmental process has been made using Actinomycin D, as described in the accompanying report (Pannbacker and Wright, 1966).

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