THE CONTENT AND RELATIVE BASE RATIOS OF RIBONUCLEIC ACID IN *AMOEBA*

RAY M. IVERSON, Ph.D.

From the Department of Zoology, Laboratory for Quantitative Biology, University of Miami, Coral Gables and the University of Gothenburg, Sweden

ABSTRACT

The amount and relative base ratios of ribonucleic acid (RNA) in the nucleus and cytoplasm of *Amoeba proteus* and *A. dubia*, and of homospecies cells obtained by nuclear transfer with *A. proteus*, have been determined by microelectrophoresis. In *A. proteus* the average amounts of RNA in the nucleus and the cytoplasm were 134. micromicrograms and 2520. micromicrograms; in *A. dubia* the averages for the nucleus and cytoplasm were 67. micromicrograms and 1427. micromicrograms. The relative base ratio of RNA of the nucleus is similar to that of the RNA of the cytoplasm within a species, but the two species differed in this respect. Homospecies nuclear transfer did not affect the relative base ratio or amount of RNA.

INTRODUCTION

Ribonucleic acid (RNA) from the nucleus (4, 13, 15, 16) has been detected in the cytoplasm (7, 10, 17, 20), where it is involved in the synthesis of cytoplasmic proteins. My current research concerns the amount and type of RNA passing into the cytoplasm under various physiological conditions, and at different stages of the cell cycle. This paper, based upon the precise techniques of microelectrophoresis (microphoresis), reports the amount and base ratios of RNA in the nucleus and cytoplasm of *Amoeba proteus*, *Amoeba dubia*, and cells obtained by homospecific nuclear transfer between different *A. proteus* cells.

MATERIALS AND METHODS

Amoeba proteus and *A. dubia* were obtained from Dr. J. A. Dawson, Floral Park, New York. *A. proteus* was grown as previously described (11), and *A. dubia* by the methods of Dr. Dawson (5). The amebae were without food for 4 days prior to nuclear transfers. Two days after nuclear transfer, the cells were fixed, and the RNA was extracted for analysis. Thus all cells were starved for 6 days prior to fixation. After 2 to 3 days the food vacuoles cannot be detected under the UV microscope at × 93.

The nucleus was separated from the cytoplasm as follows: the nucleus from a single cell, restricted to a small drop of culture medium on the dissection coverslip, was ejected from the cytoplasm with a microneedle. The nucleus and cytoplasm were then individually picked up on the needle and moved onto the extraction coverslip. Nuclei were either pooled in groups of 5 to 10 for base ratio analysis, or were separately analyzed for the total amount of RNA. (The ejection of the nucleus from the cytoplasm and its placement on the extraction coverslip usually took less than 15 seconds, and never more than 30 seconds; thus, the nucleus usually was in the inorganic culture medium outside the cell for about 5 seconds, and never longer than 15 seconds.) The samples were fixed in 70 per cent ethanol for 10 minutes, air-dried, washed with 2 per cent perchloric acid at 4°C for 10 minutes, and then washed several times in absolute ethanol, in which they were stored overnight (19). The next day they were washed in chloroform for 10 minutes, in absolute ethanol for 1 minute, and then were air-dried. Microphoresis was performed by Edström's methods (6).

RESULTS

Amount of RNA

Table I gives the amount of RNA in the nucleus, cytoplasm, and whole cell of *A. proteus*, *A. dubia*, and cells produced by homospecific nuclear transfer in *A. proteus* (N_pC_p). The analyses were of cell samples taken over a period of 4 months. *A. dubia* had approximately half as much nuclear and cytoplasmic RNA as did *A. proteus*. The nucleus and cytoplasm from the cells upon which homospecific nuclear transfers were performed (N_pC_p cells) did not show a significant change in their RNA content after 2 days. The ratio of nuclear to cytoplasmic RNA was approximately the same in these species, about 1:12.

The differences in the mean RNA content were statistically evaluated (Table II) using the t test at the 0.05 significance level (18). The null hypothesis consists of no difference, or equality, of the means of the sampled populations. In this report, $t_{.05}$ is used for the criterion of the test and t' for the value being compared with $t_{.05}$. A comparison (Table II) of the amounts of nuclear and cytoplasmic RNA in the control *A. proteus* and the N_pC_p cells shows no significant difference because t' is smaller than $t_{.05}$. Thus, nuclear transfer did not apparently affect the amount of RNA of the *A. proteus* cell.

RNA Base Ratios

Table III presents the relative base ratios of RNA (in per cent of the sum) of the nucleus, the cytoplasm, and the whole cell of *A. dubia*, *A. proteus*, and N_pC_p cells. Within each species the nucleus and the cytoplasm had approximately the same relative base ratio of RNA, but the two species differed in this respect. The truly significant difference procedure (see reference 18, p. 109) shows a clear distinction between the base ratios of *A. dubia* and those of *A. proteus* and the N_pC_p cells. Homospecific nuclear transfer in *A. proteus* (N_pC_p cells) did not result in a significant alteration of the relative base ratio of RNA in the nucleus or the cytoplasm (Table III). Thus, any disturbance in the RNA resulting from the process of nuclear transfer appears to be either negligible, quickly repaired, or not detectable by microphoresis.

TABLE I

Mean Amount of RNA in Amoeba

Material	Number of analyses	Micromicrograms of RNA
A. proteus		
nucleus	32	134
cytoplasm	25	2520
whole cell	42	2589
A. dubia		
nucleus	29	67
cytoplasm	63	1427
whole cell	8	1419
N_pC_p*		
nucleus	27	132
cytoplasm	57	2299

* Homospecific nuclear transfers on *A. proteus* cells.

TABLE II

Statistical Comparison at 0.05 Significance Level of Amounts of RNA in Amoeba Cells

	$t_{.05}$	t'
Nucleus of control *A. proteus* compared with nucleus of N_pC_p	2.044	0.221
Cytoplasm of *A. proteus* compared with cytoplasm of N_pC_p	2.04	1.09
Nucleus of *A. proteus* compared with nucleus of *A. dubia*	2.044	6.828

DISCUSSION

As determined by microphoresis, the amount of RNA per *A. proteus* cell was 2589. micromicrograms. This amount was between the value of 1000. micromicrograms per cell, reported by James (12), and 6800. micromicrograms, reported for this species by Brachet (1). These differing values probably result from the different methods of analysis and from variation of a population. Variation within a population can be wide; the cytoplasm of each of 50 cells from one culture was individually analyzed and the RNA was found to vary from 813. to 3680. micromicrograms, with a mean of 1990. micromicrograms (Iverson, unpublished observations).

In *A. proteus* the relative base ratios of RNA, for adenine and uracil, but not for guanine and cytosine, agreed with those reported by Cummins and Plaut (3). In my experiments, the amount of

TABLE III

Relative Base Composition of RNA from Amoeba

Material	No. of analyses	Adenine			Guanine			Cytosine			Uracil		
		Base ratio	SDM*	CV‡	Base ratio	SDM	CV	Base ratio	SDM	CV	Base ratio	SDM	CV
A. proteus													
Nucleus	20	20.2	(0.62	13.8)	35.2	(0.62	7.9)	24.1	(0.55	10.1)	20.0	(0.62	13.8)
Cytoplasm	72	19.9	(0.29	12.4)	34.5	(0.39	9.6)	26.9	(0.48	15.2)	18.8	(0.31	14.0)
Whole cell	24	21.0	(0.46	10.7)	36.0	(0.59	8.0)	24.1	(0.54	10.9)	18.9	(0.58	14.9)
A. dubia													
Nucleus	16	25.7	(0.64	10.0)	31.7	(0.57	7.2)	21.0	(0.62	11.8)	21.7	(0.74	13.6)
Cytoplasm	65	24.0	(0.66	22.1)	33.7	(0.49	11.8)	20.7	(0.54	21.1)	21.6	(0.50	18.8)
Whole cell	31	23.1	(0.51	12.3)	35.1	(0.47	7.5)	18.7	(0.54	16.1)	23.2	(0.54	12.9)
N_pC_p§													
Nucleus	7	20.3	(0.009	0.11)	35.0	(0.73	5.5)	25.6	(1.22	12.7)	19.0	(0.84	11.7)
Cytoplasm	27	21.0	(0.71	17.6)	34.6	(0.78	11.7)	25.6	(0.85	17.2)	19.4	(0.73	19.6)

* Standard deviation of mean.

‡ Coefficient of variation.

§ Homospecific nuclear transfers on *A. proteus* cells.

guanine was higher relative to the other three bases, whereas Cummins and Plaut report relatively more cytosine. Preliminary RNA base ratios determined microphoretically by Tencer and Edström (19) on the Brussels strain of *A. proteus* were similar to those reported here.

Within each species the similarity between the relative base ratios of RNA of the nucleus and cytoplasm may be accounted for by the presence of numerous nucleoli (2, 9) and intranuclear dense bodies (8, 14) within the *Amoeba* nucleus. It seems unlikely that this similarity in RNA base ratios is a result of diffusion of RNA out of the nucleus during the approximately 5 seconds it was in the small drop of culture medium after being ejected from the cytoplasm. The nucleus retained its shape and size well after prolonged exposure (1 hour) to the culture medium, and preliminary investigations indicate that little ultraviolet-absorbing material was lost from the nucleus under the experimental conditions.

Since the amount and relative base ratios of RNA differ between cells of *A. proteus* and *A. dubia*, and since they were not altered by the process of nuclear transfer, I am utilizing these differences for investigating nuclear and cytoplasmic events following interspecies nuclear transfers.

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