COMPOSITION OF RIBONUCLEIC ACID FROM VARIOUS PARTS OF SPIDER OOCYTES

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ABSTRACT

Microphoretic purine-pyrimidine analyses of the ribonucleic acid (RNA) in nucleoli, nucleoplasm, cytoplasm, and yolk nuclei of spider oocytes have been carried out. The material necessary for the analyses was isolated by micromanipulation. Determinations of the amounts of RNA in the different parts of the cell were also performed. No differences between the composition of RNA in the nucleolus and the cytoplasm could be disclosed. Nucleoplasmic RNA was, on the other hand, distinctly different from that in the nucleolus and in the cytoplasm. The difference lies in the content of adenine, which is highest in nucleoplasmic RNA. The few analyses carried out on yolk nuclei showed their RNA to be variable in composition with a tendency to high purine values. The cytoplasm contains about 99 per cent of the total RNA in these cells, the nucleoplasm about 1 per cent, and the nucleolus not more than 0.3 per cent, although the highest concentrations are found in these latter structures. When considered in the light of other recent findings the results are compatible with the view that nucleolar RNA is the precursor of cytoplasmic RNA.

While the nucleotide composition of RNA in cytoplasm and nuclei is known in several cases, there is a scarcity of data on the nucleolar RNA and a complete absence of such for nucleoplasmic RNA (nucleoplasm being the non-nucleolar nuclear material). Consequently it has been impossible to compare the composition of RNA in the three cell regions, nucleoplasm, nucleolus, and cytoplasm, for the same type of cell. The present report describes microphoretic nucleotide analyses carried out on individually isolated parts of spider oocytes. The RNA in the cytoplasm, nucleolus, and nucleoplasm was investigated. A few analyses were also performed on RNA from yolk nuclei.

MATERIALS AND METHODS

As is evident from much work on isolated cellular constituents, losses of RNA may occur during the isolation, unless special precautions are taken, such as lyophilizing the tissue and performing the isolation in non-aqueous media. Another possibility is to precipitate the RNA *in situ* with acid ethanol, *e.g.* with Carnoy fixation, and carry out the isolation afterwards. This alternative can probably not be used for bulk isolation because of the hardening of the tissue constituents but is practicable when the desired parts can be isolated individually by microdissection. This method was used in the present work.

The ovaries from six specimens of the common house spider (*Tegenaria domestica*), collected from June to September, were fixed with the rest of the contents of the abdomen in Carnoy's fluid (ethanol, chloroform, and concentrated acetic acid, 6:3:1, by volume) for 1 hour, after which they were transferred *via* absolute ethanol and benzene to paraffin. The embedded organs were cut at 7 μ for staining and 20 μ for the chemical investigations. For localization of RNA in control sections, staining was performed

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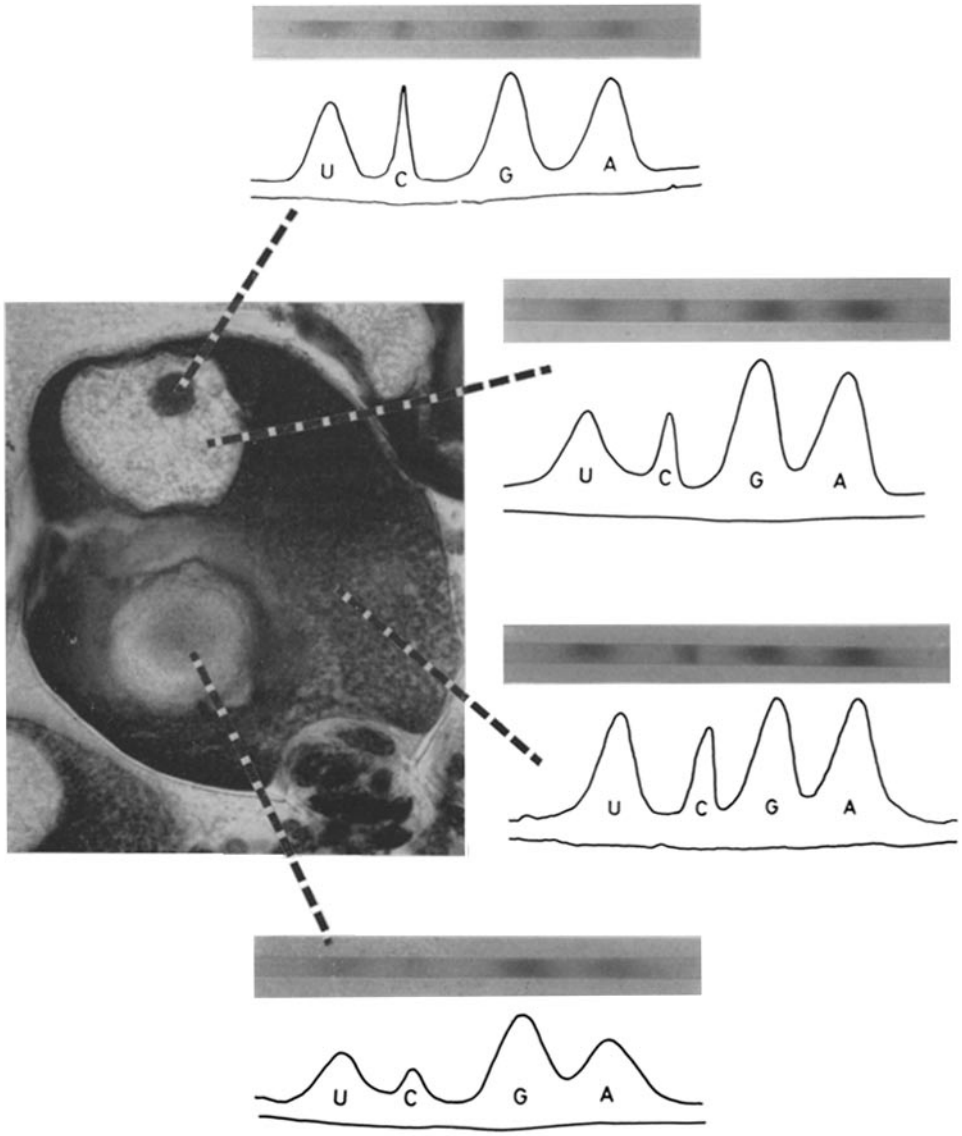

FIGURE 1

To the left a microphotograph of an oocyte from the spider, stained with methylene blue. Dark areas represent high concentrations of RNA. The cell nucleus with the nucleolus is seen in the upper part, and the yolk nucleus in the lower part of the cell. Representative microphoretic analyses (from other cells of the same kind), shown to the right, consist of photographs in ultraviolet light at 257 mμ and photometer curves. *A*, *G*, *C*, and *U* stand for adenine, guanine, cytidylic acid, and uridylic acid, respectively. The cathode is to the right and the starting point close to *U*, between *U* and *C*. Magnification: oocyte, × 500, separations, × 100.

TABLE I

RNA Concentration and Content in Different Parts of Tegenaria Oocytes of a Diameter around 120 μ

	RNA (*w/v*)	Volume	RNA	Per cent of the total RNA
	Per cent	μ^3	$\mu\mu g.$	
Nucleolus	7.9; 7.8	1,250	100	0.3
Nucleoplasm	1.0; 1.0	30,000	300	1
Cytoplasm	3.0; 4.3	900,000	30,000	99

according to Pischinger (18). The Feulgen reaction was carried out on some sections. The ovaries were also investigated in the Köhler ultraviolet microscope at 257 mμ before and after ribonuclease digestion.

For purine-pyridine analysis, the embedded sections, mounted on coverslips, were deparaffinized and hydrated with 0.01 N acetic acid. Sections of large oocytes in stages devoid of visible yolk (80 to 150 μ diameter) were isolated as described earlier (4) in an oil chamber using de Fonbrune's micromanipulator equipped with two needles. Nucleoli, recognizable by their light-refracting properties and relatively solid consistency were manipulated out of surrounding nucleoplasm and freed from it. Nucleoplasm free from nucleoli was loosened from the inside of the nuclear membrane and collected from several cells for analysis. Small pieces of cytoplasm were taken anywhere in cells from which other parts had been collected. Yolk nuclei with the outer shells removed to eliminate the danger of cytoplasmic contamination were also prepared. Consequently the values found for these may not be representative for whole yolk nuclei.

Collections of cell parts from about 20 cells were extracted and analyzed together. Five to 10 analyses were carried out on the RNA extracted from each such collection. The extractions were performed in the oil chamber and the extracted RNA was analyzed by microphoresis according to the standard procedure (6). Some determinations of RNA content and concentration were also made using the author's method (4), in which the RNA extracted from microscopic tissue units is determined in round drops by a photographic-photometric procedure in ultraviolet light. In order to obtain volume values on isolated cell pieces, three diameters were measured. This is by no means an exact method, but in the present cases it was only of interest to get round figures for the RNA content and concentration in the different cell parts.

RESULTS

Oocytes in stages prior to visible yolk formation show the highest concentrations of RNA in the nucleoli (about 8 per cent, *w/v*). The concentration in the cytoplasm is about half as high, and that of the nucleoplasm only 1 per cent. Because of the volume ratios, however, the nucleolus contains the smallest amounts of RNA (Table I).

The Feulgen reaction is negative for oocyte nuclei (dilution effect) as well as for cytoplasm. Other, smaller nuclei in the sections are positive.

The results of the microphoretic analyses are given in Table II and Fig. 1 is an illustration to the results. The nucleolar and cytoplasmic RNA do not differ statistically in composition. The RNA from nucleoplasm differs on the other hand markedly from that of cytoplasm ($P < 0.003$) and nucleoli ($P < 0.001$) with regard to adenine

TABLE II

Purine-Pyrimidine Composition of RNA from Different Parts of Tegenaria Oocytes

Mean values of molar proportions in per cent of the sum, ± S.E.M.

	Adenine	Guanine	Cytosine	Uracil	$\frac{\text{Adenine}}{\text{Cytosine}}$	$\frac{\text{Purines}}{\text{Pyrimidines}}$	No. of animals	No. of analyses
Nucleolus	25.2 ± 0.2	29.8 ± 0.9	22.9 ± 1.0	22.2 ± 0.7	1.10	1.22	6	36
Nucleoplasm	28.4 ± 0.6	28.5 ± 1.3	20.3 ± 0.8	22.7 ± 0.5	1.40	1.32	5	36
Cytoplasm	25.1 ± 0.5	30.2 ± 0.5	21.9 ± 0.6	22.9 ± 0.7	1.15	1.23	6	37
Yolk nucleus	28.4	33.5	19.5	18.7	1.46	1.62	3	17

content. Nucleoplasmic RNA contains more adenine and less cytosine than the other types of RNA. Guanine and uracil showed the same percentages in both types of RNA and consequently the ratio between 6-amino and 6-keto compounds was the same. Yolk nuclei were only analyzed in three animals. Their RNA showed a variable composition and was found to be relatively rich in purines, particularly guanine.

DISCUSSION

The present analyses show that, with the technique used, the nucleolar and cytoplasmic RNA are undistinguishable. A difference in composition as large as the one found by Vincent (23) for starfish oocytes would easily have been detected. The question is whether the discrepancy is due to species variation or to artefacts during the preparations. As shown by Vincent in a later paper (24) nucleolar RNA is partly lost during macroscale isolation, which might give non-representative values. Judging by the conditions in other tissues RNA is preserved during controlled Carnoy fixation (see Edström, 5, for discussion). Thus it seems likely that the present results are representative of the *in vivo* status.

It is known that there is a qualitative difference between nuclear and cytoplasmic RNA (7), for further reference see Magasanik (17). Nuclear RNA is largely nucleoplasmic (75 to 80 per cent according to Table I and Johnston *et al.*, 16). These data are in good agreement with the fact that the present results show a qualitative difference between nucleoplasmic and cytoplasmic RNA.

Harris (15) obtained indirect evidence from autoradiographic experiments that nucleolar and nucleoplasmic RNA differ qualitatively, the quotient between adenine and cytosine being higher in the nucleoplasm. Such a difference has been directly demonstrated in the present investigation.

Goldstein and Plaut (13) and Goldstein and Micou (11) have shown a transfer of nuclear RNA to the cytoplasm in the amoeba and in cultured human amnion cells. Prescott (19) found that in the amoeba there is "a complete dependence on the nucleus for RNA synthesis." For cultured connective tissue cells on the other hand Harris (15) demonstrated that only a small part of the nuclear RNA can be the precursor of cytoplasmic RNA. If a general mechanism exists, one way of interpreting these findings, in the light of the present results, would be that nucleolar RNA is the precursor of cytoplasmic RNA (in itself not a new idea), while nucleoplasmic RNA represents a different system.

The view advanced here does not exclude the possibility that the synthesis of nucleolar RNA occurs in the nucleus outside the nucleolus (22, 12). In such a case the nucleoplasmic RNA is either heterogeneous with respect to nucleotide composition and contributes to the nucleolar RNA to a varying extent for different fractions, or the nucleolus modifies the nucleotide composition of the RNA which passes through it. The nucleoplasmic RNA seems to be largely chromosomal in oocytes (2, 10). Evidence for differential synthetic activity along giant chromosomes has been found (1, 3). It has been shown that not only DNA (9, 20, 21) but also RNA (14, 10) is formed in this process. In the lampbrush chromosomes of oocytes the loops have been found to synthesize RNA actively (2, 10). These findings speak in favour of the former alternative. The only argument in favour of the latter is that it would give the nucleolus an obvious functional importance.

In any case, it might seem difficult to reconcile the idea that all cytoplasmic RNA comes from the nucleolus with the fact that the RNA amounts in the cytoplasm according to the present findings are about 300 times larger than those of the nucleolus. However, Ficq (8) has shown for starfish oocytes, that the nucleolus may incorporate RNA precursors to an extent that is about 100 times higher than the incorporation in the cytoplasm and consequently may be able to compensate for small amounts of RNA with a high rate of turnover.

The few analyses carried out on isolated yolk nuclei gave variable results with a general tendency for a high content of purines. This fact together with the observation made during the microdissections, that these structures are extremely dense, does not suggest a very active role for them in cellular metabolism.

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