NUCLEOLAR RNA COMPOSITION DURING GROWTH OF THE STARFISH OO[illegible]E

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Vincent (1952) demonstrated pronounced differences in base composition between RNA from nucleoli and cytoplasm in mature starfish oocytes. Differences in the same direction although of a smaller size were found by Edström *et al.* (1961) in analyses of younger, but relatively large oocytes. It might seem difficult to reconcile these findings with all the evidence for a nucleolar origin of ribosomal RNA (McConkey and Hopkins, 1964, for ref.), which constitutes the main RNA fraction in the cytoplasm. It is obvious, however, that the nucleolar RNA found in full grown oocytes cannot give a substantial relative contribution in the cytoplasm on account of the imminent disappearance of the nucleolus. If there are base changes of the nucleolar RNA during the end of the life of the nucleolus, these consequently cannot be expected to be reflected to any larger extent in the composition of the cytoplasmic RNA, the ribosomal component of which probably does not turn over (Graham and Siminovitch, 1957; Davern and Meselson, 1960; McMaster-Kaye, 1960). During earlier growth stages one would, on the other hand, expect a better agreement. In order to test this hypothesis microelectrophoretic RNA base analyses were carried out on nucleoli and cytoplasm isolated from starfish oocytes in early and late growth stages.

MATERIALS AND METHODS: Small pieces of ovaries were removed from specimens of the starfish Asterias rubens obtained in November and March. The pieces were fixed in Carnoy's solution for 90 min, transferred to absolute ethanol, benzene and embedded in paraffin. Sections, 25 μ thick, were cut and spread on coverslips, deparaffinized and hydrated with 0.01 N acetic acid. A coverslip was placed in an oil chamber and pieces of cytoplasm and whole nucleoli were isolated by micromanipulation. Samples of non-nucleolar nuclear material (nucleoplasm) were collected for comparison. RNA was extracted from isolated parts with a buffered ribonuclease solution. It has been demonstrated for this tissue 1) that RNA is quantitatively preserved up to the stage of ribonuclease extraction, 2) that free nucleotides are efficiently removed prior to this stage, and 3) that RNA is completely extracted during the digestion with a selectivity good enough for the analytical procedure (Edström et al., 1961). The RNA extracts were evaporated to dryness, dissolved in 4 N hydrochloric acid and hydrolyzed in a micropipette. The hydrolysates were finally analyzed by microelectrophoresis in a microscopic cellulose fiber (Edström, 1960).

RESULTS AND CONCLUSIONS: Data were collected from one group of four animals with young oocytes, having a diameter after fixation of 45-55 μ. A second group consisted of tissue from three animals with large oocytes of a fixed cell diameter of 80-110 μ. From each animal 3 to 5 analyses were run and the mean values are given in Table I. It can be seen that for the young cells there is in all cases a striking similarity between the nucleolar and cytoplasmic RNA. For the older cells, on the other hand, the nucleolar RNA composition differs markedly from that of the cytoplasm, although in no case to the extent found by Vincent (1952). Nucleoplasmic RNA is very unlike that of the other

Table I

The Base Composition of Nucleolar and Cytoplasmic RNA in Young and in Large Starfish Oocytes

Young oocytes diam. 45-55 μ	Animal no.	adenine	guanine	cytosine	uracil
Nucleoli	I	21.8	30.6	27.8	19.9
	II	22.9	32.1	24.7	20.2
	III	23.2	32.0	24.4	20.5
	IV	23.0	31.4	26.0	19.4
	mean	22.7	31.5	25.7	20.0
Cytoplasm	I	24.1	30.2	25.7	20.0
	II	23.6	30.1	26.2	20.1
	III	23.1	32.8	23.7	20.6
	IV	22.6	31.1	25.4	20.3
	mean	23.4	31.1	25.3	20.3
Large oocytes diam. 80-110 μ	**Animal no.**	**adenine**	**guanine**	**cytosine**	**uracil**
Nucleoli	V	23.7	33.4	24.3	18.5
	VI	22.2	34.1	27.5	16.2
	VII	24.3	33.9	24.1	17.7
	mean	23.4	33.8	25.3	17.5
Cytoplasm	V	23.5	31.9	24.8	19.7
	VI	23.6	30.9	27.1	18.5
	VII	24.9	31.7	24.9	18.5
	mean	24.0	31.5	25.6	18.9

two components and shows great variations from animal to animal (Table II).

The present results are compatible with the notion that the larger part of the cytoplasmic RNA derives from the nucleolus, since differences in base composition are found only in late growth stages when the nucleolus is about to disappear. The cause of the change in nucleolar RNA composition is unknown and may be the result either of changed proportions between different RNA fractions, known to exist here (Vincent, 1954; Sibatani *et al.* 1962; Adams and Busch, 1962), or of

Table II

The RNA Composition in the Non-nucleolar Nuclear Compartment (Nucleoplasm) in Starfish Oocytes

Animal no.	adenine	guanine	cytosine	uracil
I	27.1	22.3	24.6	25.9
II	31.6	24.4	18.0	26.0
III	28.5	22.0	26.9	22.6
IV	28.3	25.6	22.2	23.8
V	35.7	24.8	16.5	23.0
VI	38.8	21.4	20.2	19.6
VII	39.9	17.9	17.6	24.6

degradative changes in the nucleolar RNA, setting in before its dissolution. The agreement between cytoplasmic and nucleolar RNA in early stages contrasts drastically with the variable RNA of the nucleoplasm, which shows little resemblance to that of the other two components. If ribosomal RNA is not turning over in the growing oocyte a contribution of nucleolar RNA of a changed composition in the later stages may be expected to influence the cytoplasmic RNA only moderately, being diluted by pre-existing RNA. A slight increase of the cytoplasmic guanine/uracil quotient is nevertheless found with age, whether this is due to the nucleolar change or has other causes.

SUMMARY: The base composition of nucleolar RNA from young and large (near maturity) starfish oocytes differs, the latter RNA having a higher guanine/uracil ratio. Only in young oocytes has the nucleolar RNA the same composition as cytoplasmic RNA, which changes only slightly during development. Nucleoplasmic RNA shows no resemblance to the other two kinds of RNA.

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