RIBONUCLEIC ACID IN THE OOCYTES OF THE ASCIDIAN *PYURA* SP. (PYURIDAE : PLEUROGONIA)

by B. R. SESHACHAR, F.N.I. *and* S. R. V. RAO, *Department of Zoology, University of Mysore, Central College, Bangalore*

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ABSTRACT

The 'test cells' are characteristic of the ascidian ovum. In *Pyura* sp., they do not occur in the early oocyte but appear much later in its peripheral cytoplasm. They are rich in ribonucleic acid. In later stages of oogenesis, they break up and release their RNA content into the cytoplasm of the developing oocyte. In addition to the nucleolus and the oocyte cytoplasm, which are important sources of RNA, the test cells form a third source. Cytochemical reactions indicate that the test cell RNA is different from that of the nucleolus and oocyte cytoplasm.

INTRODUCTION

One of the basic facts of modern cytology and cell physiology is the establishment of an undeniable association between ribose nucleic acid (RNA) and protein synthesis in the animal cell. Independently put forward by Caspersson (1941) and Brachet (1942) this view has gained increasing support in view of the evidence that has accumulated in recent years. Recently, Brachet (1957) has reviewed the problem and has summarized the experimental evidence in favour of it.The origin of the RNA in the cell, however, has been the subject of debate and at least two sources are cited, (a) the microsomes in the cytoplasm and (*b*) the nucleolus in the nucleus. Both are rich in RNA and evidence that either or both are sources of its origin has accumulated during recent years (Brachet, 1957). One of the most striking is that presented by Fauré-Fremiet *et al.* (1950) who, in their studies of the developing oocytes of *Glomeris* have clearly established this dual origin of RNA. It is more or less clearly understood that whenever synthesis and growth are taking place in the cell, abundant amounts of RNA are present in the cytoplasm and in the nucleus (in the nucleolus). Other possible sources of RNA have been occasionally investigated but have not been established. In this context, the ascidian egg offers special problems. The "inner follicle cells" or "test cells" of the ovum are peculiar to the ascidian and their role has not been established. Berrill (1950) states, "The inner follicle cells, often called by the ambiguous term "test cells", have long been the subject of controversy with regard to the role they play in the growth of the ovum" (page 36).

Recent examination of oogenesis in an Indian ascidian *Pyura* sp. (Pyuridae Pleurogonia) has offered some ideas which are presented in this paper.

MATERIAL AND METHODS

The material used in the present study was collected in January 1958 at Madras. The gonads were fixed in Carnoy's and Sanfelice's fluids. They were cut at varying thickness and stained with the Feulgen reagent and counterstained with light green. Methyl green-pyronin and toluidine blue were the other stains employed. Digestion with trichloracetic acid and ribonuclease was also done in order to establish the areas of presence and distribution of RNA.

*Present address : Cancer Research Institute (W.I.A.) Gandhinagar, Madras-20.

Observations

The Ooocyte : Examination of young oocytes (up to 60–75μ diameter) shows that their cytoplasm is rich in RNA as evidenced by its strong affinity for both toluidine blue and pyronin (Pl. VIII, fig. 1). One of the striking features of this early development is the gradual increase in size of the nucleolus which reaches a maximum diameter of 10μ when the cell is 60μ, after which no further increase appears to take place (Rao, 1959). In later stages of growth, when the oocyte attains a diameter of 75μ or more, there is a gradual decrease in cytoplasmic basophilia. When the oocyte reached a diameter of 90–120μ there appears a peripheral zone in the cytoplasm exhibiting strong basophilia (Pl. VIII, fig. 3). Extraction with ribonuclease and hot trichloracetic acid confirms that this zone is rich in RNA. This peripheral zone is also rich in mitochondria.

The Test Cells : These are characteristic of the ascidian oocyte and occur in its periphery. They are rounded cells with small nuclei (Text fig. 1 and Pl. VIII, fig.2). They seem to project into the cytoplasm of the oocyte but are distinct from it and they possess a clear cell membrane. During early stages of the development of the oocyte, i.e. when its diameter is less than 60μ, the test cells are few and inconspicuous. Later, they grow larger and form characteristic structures of the oocyte. Their cytoplasm gradually accumulates basophilic material in the form of large granules which fill the entire cell. The basophilia is so intense that only in Feulgen preparations or in those subjected to extraction by ribonuclease or hot trichloracetic acid can the nucleus bes een (Pl. VIII, fig. 2). The nucleus is small and has a number of coarse chromatin granules. Sometimes the test cells migrate into the cytoplasm of the oocyte, and occasionally several test cells are seen close to the nucleus (Text fig. 1).

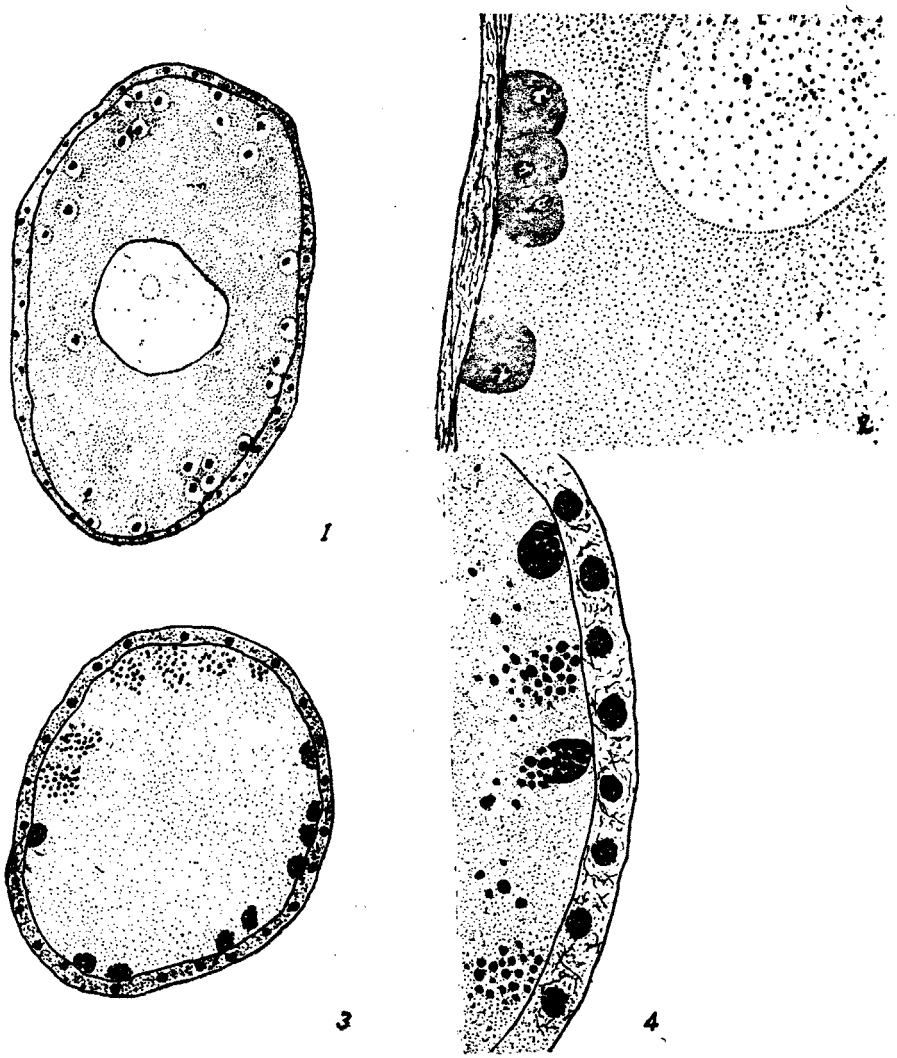
Treatment with ribonuclease and hot trichloracetic acid (see Seshachar, 1950, for method) shows that the basophilia of the oocyte cytoplasm as well as that of the nucleolus becomes considerably diminished, and on prolonged treatment, the basophilia is lost altogether. On the other hand, the basophilia of the cytoplasm of test cell is not extracted easily. In preparations stained with toluidine blue after extraction with 5 per cent trichloracetic acid at 80°C for 15 minutes, the oocyte cytoplasm and nucleolus are perfectly clear, but the test cells are still dark though they are not so dark as to obscure the nucleus (Text fig. 2). Extraction with ribonuclease is even slower. After one hour's digestion, the cytoplasm and nucleolus are clear but the test cells exhibit basophilia to a relatively high degree. Only prolonged extraction with trichloracetic acid at 90°C removes basophilia altogether from the test cells.

The later fate of the test cells is interesting. In oocytes of diameter of 90μ–120μ, the test cells are seen to break up and release their basophilic granules into the peripheral cytoplasm of the egg (Text figs. 3 & 4). The intense basophilia noticed in the egg periphery at this stage is almost entirely due to this. Stages in this disintegration are quite easily seen. The nucleus also disintegrates later. Older oocytes do not show the test cells in their cytoplasm.

Discussion

In the developing egg of *Pyura*, there are at least three sources of RNA. In early stages, the intense basophilia of the nucleolus and cytoplasm indicates the presence of ribonucleotides in these two cell constituents. It was pointed out earlier (Rao, 1959) that the growth of the nucleolus reaches a maximum when the oocyte diameter is about 60μ, and thereafter remains stationary. At a slightly later stage when the oocyte diameter is about 57μ, the cytoplasmic basophilia starts declining. Again, when the oocyte reaches a diameter of 90μ–120μ, there appears a ring of intensely basophilic granules in its periphery. Apparently in the early

stages of growth, the source of RNA is the nucleolus and the general cytoplasm. Later stages are characterised by a third source, *i.e.* the test cells, which have all along been accumulating RNA in their cytoplasm and which now break up releasing their contents into the cytoplasm of the oocyte.



TEXT-FIG. 1.

Fig. 1. Oocyte of *Pyura* sp. showing 'test cells' in the cytoplasm Feulgen. ×1260.
Fig. 2. Part of oocyte of *Pyura* treated with 5% Trichloracetic acid at 80°C for 15 minutes and stained with toluidine blue. The oocyte cytoplasm is clear but the test cells show basophilia. ×2800.
Fig. 3. Oocyte at a later stage with the test cells breaking up and releasing their basophilic granules into the cytoplasm. Toluidine blue staining after ribonuclease treatment. ×840.
Fig. 4. Part of the same showing the large, coarse granules in the peripheral cytoplasm of the oocyte. Toluidine blue staining after ribonuclease treatment. ×1850.

There is reason to believe that the RNA of the test cells is in some way different from that of the oocyte nucleolus and cytoplasm. Its reactions to trichloracetic acid and ribonuclease would indicate this. Vincent (1957) has recently provided evidence of a heterogeneity of RNA in the starfish oocyte, and it is possible that in *Pyura* also, the RNA is of more than one kind.

Acknowledgement

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Explanation of Figures in plate VIII

Fig. 1. Section of ovary of *Pyura* sp. stained with toluidine blue. The more pronounced basophilia of the younger oocytes is clearly seen. ×100.

Fig. 2. Oocyte showing test cells in its periphery. The young oocyte in the lower left corner has none. Ribonuclease treated. Toluidine blue. ×350.

Fig. 3. Part of oocyte at a later stage showing peripheral accumulation of intensely basophilic granules.


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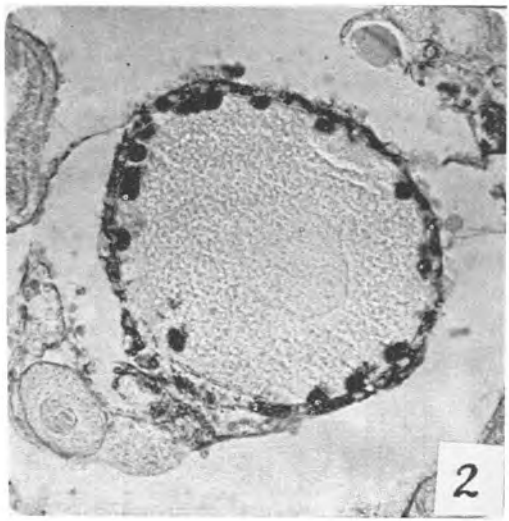
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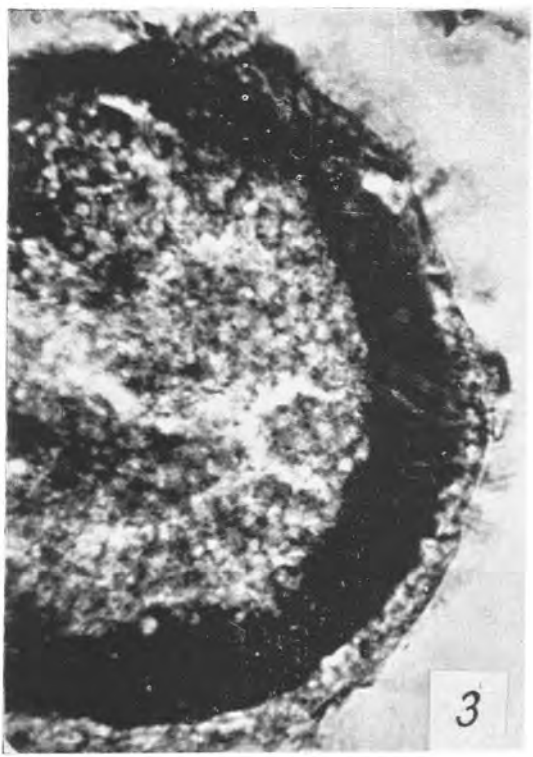
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