A Cytochemical and Autoradiographic Study of Oocyte Nucleoli in *Limnaea stagnalis* L.

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Summary. In growing oocytes of *Limnaea stagnalis* two nucleoli: a smaller nucleolus—paranucleolus, and a larger one—eunucleolus, are found. At an early stage of previtellogenesis both nucleoli stain homogeneously; at a later stage the eunucleolus becomes vacuolated. In the course of vitellogenesis the eunucleolus develops into an amphinucleolus. The paranucleolus does not disappear during previtellogenesis, and it appears to be still present during vitellogenesis. During the final growth phase of the oocyte the two nucleoli unite and then disintegrate.

Cytochemical studies indicate that in each of the nucleoli basic proteins, non-histone proteins and RNA are present. Incorporation of $^3$H-uridine occurs only in the eunucleolus. The paranucleolus does not show RNA metabolism. The amphinucleolus consists of two component parts differing in their origin and cytochemical composition. The primary part of the nucleolus is rich in proteins and RNA. The secondary part, which forms as a result of the secretory activity of the nucleolus, contains only proteins.

The paranucleolus shows a considerable similarity to the endobody found in insect oocytes.

Key words: Nucleoli — Oocytes — *Limnaea stagnalis* — Type of nucleoli — Cytochemistry, Autoradiography.

Introduction

In recent years there have been many studies concerned with the morphology, ultrastructure, cytochemistry and functions of the nucleolus (Busch and Smetana, 1970). These studies indicate that the role of the nucleolus in the biogenesis of the ribosomes is of primary importance. Although the main constituent of the nucleolus is protein, the metabolism of the proteins in the nucleolus is little known. The metabolism of RNA is known much better.

The amphinucleolus, found in the oocytes of molluscs, is a particular kind of nucleolus. Many authors analysed the nucleoli in the growing oocytes of molluscs, for instance Bretschneider and Raven (1951), Ranzoli (1953), Bolognari (1956, 1959), Bolognari and Cannata (1963), Yamamoto and Yamada (1960), Yamamoto (1966), Romanova (1963, 1964, 1970), Romanova and Gazarian (1966), Ubbels (1968), Bottke (1973).

In young oocytes of *L. stagnalis* two nucleoli are found, a smaller one—paranucleolus, and a bigger one—eunucleolus (Bretschneider and Raven, 1951; Ubbels, 1968). According to these authors the paranucleolus disappears during late previtellogenesis, while the eunucleolus becomes vacuolated. During vitellogenesis the eunucleolus transforms into an amphinucleolus. At the end of vitellogenesis the amphinucleolus disappears, too.

According to Bretschneider and Raven (1951), initially, the eunucleolus shows acidophil properties, and is basophilic at the end of the growth phase of the oocyte.
A change in the affinity to stains was also recorded by Ubbles (1968). Her studies indicate that the basophilic constituents of the eunucleolus decrease, and as a result the nucleolus shows an affinity to acid stains.

The eunucleolus shows clear signs indicating synthetic activity. Two types of nucleolar product formation can be distinguished (Bretschneider, 1946, quoted after Ubbels, 1968). Bretschneider and Raven (1951) distinguish intranucleolar product formation, and epinucleolar product formation. Both types occur in one species, but then usually at different oogenetic stages (Raven, 1961).

The contradictory data found in the literature inclined us to analyse the nucleoli during the oocyte growth in *L. stagnalis*, using cytochemical methods. We supplemented our observations with autoradiographic investigations.

**Materials and Methods**

The object of the investigations was the ovotestis of *L. stagnalis* (ponds at Milicz). The ovotestis to be used for cytological investigations was fixed in Smith's fluid and stained by Selman and Pawsey's method (1965). In this method 3% safranine solution in 50% ethanol, and 1% solution of fast green FCF in 85% ethanol are used. The method has been developed for the yolk-plates of *Xenopus laevis*. As safranine and fast green appeared to stain also nucleoli in *Xenopus* cells, we have used it successfully for the analysis of the nucleoli in *L. stagnalis*.

The material to be used for the detection of DNA, RNA, proteins (active NH, NH$_2$ and SH groups, and amido-azole groups), and basic proteins, was fixed in buffered formalin of pH 6.9. DNA was detected by Feulgen's method. RNA was stained with azure B at pH 4 (Flax and Himes, 1952). For the detection of proteins Schiff's reagent was used after previous treatment with acrolein (Duijn, 1961, according to Pearse, 1968). Basic proteins (histones) were stained after nucleic acid hydrolysis in fast green FCF at pH 8.1 (Alfert and Geschwind, 1953), amido black 10 B at pH 8.1 (Erenpreiss, 1965), and bromphenol blue at pH 8.1. Non-histone proteins were detected after nucleic acid and basic proteins, fast green FCF of pH 4.6 was used (Kaye and McMaster-Kaye, 1966).

*L. stagnalis* ovotestis to be used for autoradiographic investigations was incubated in a modification of Ringer's solution of pH value 8.8 (Gatenby, 1934), which contained $^3$H-uridine at a concentration of 50 μC/ml. The incubation lasted from 30 minutes up to 3 hours. The material was fixed in 96% ethanol and glacial acetic acid in the proportions 6:1. The sections were coated with Ilford K2 emulsion, and exposed for 3-4 weeks.

The material was imbedded in paraffin for serial sections (7 μ thick).

**Results**

In *L. stagnalis* the oocytes develop from elements of the germinal epithelium ovotestis. Bretschneider and Raven (1951) distinguish three periods in the development of the oocyte: premeiosis, previtellogenesis and vitellogenesis. Ubbels (1968) further subdivides the period of previtellogenesis into two stages: the first and the second stages, and the period of vitellogenesis—into two more stages: the third and the fourth stages. The present investigations concern the nucleoli during previtellogenesis and vitellogenesis. In the description of the results we have used the classification introduced by Ubbels.

*Cytological Investigations*. We analysed the nucleoli by using Selman and Pawsey's method (1956). During previtellogenesis (stage 1) two nucleoli are present in the oocyte nucleus: a larger nucleolus—the eunucleolus, and a smaller one—the paranucleolus. Both these nucleoli show an affinity to safranine. The