# EVIDENCE FOR RNA IN THE HEAVY BODIES OF SEA URCHIN EGGS

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## INTRODUCTION

Ultrastructural studies on sea urchin eggs (Afzelius, 1957; Harris, 1967, 1969; Verhey and Moyer, 1967; Bal et al., 1968) have revealed certain cytoplasmic bodies, called heavy bodies, which originate from the egg nucleus. These structures are aggregations of approximately 150 A electron-opaque granules surrounded by annulate lamellae. It has been suggested that heavy bodies are cytoplasmic sites of stored nuclear developmental information. Harris (1967, 1969) has postulated that heavy bodies might be the site of ribosome-bound or inactive messenger RNA. Bal et al. (1968) have suggested that annulate lamellae might contain the information needed for the synthesis of ribosomes; therefore the heavy bodies would be packets of newly synthesized ribosomes. Other functions postulated for the annulate lamellae which might also involve heavy bodies are storage of materials for the initiation of cleavage furrow formation (Tilney and Marsland, 1969) or for membrane synthesis during embryogenesis (Anderson, 1969).

The postulated functions for heavy bodies have been based entirely on electron microscope observations of their formation and behavior in both the unfertilized and fertilized egg and on Afzelius's (1957) observation of an intense basophilic staining reaction (presumably due to RNA) corresponding to the position of heavy bodies in centrifuged sea urchin eggs. Since heavy bodies have not been isolated from homogenates of sea urchin eggs, no direct biochemical analyses have been conducted. Information concerning the chemical composition of heavy bodies, and especially the presence of RNA, has now been obtained by employing cytochemical techniques and is presented here.

## MATERIALS AND METHODS

The sea urchins, *Arbacia punctulata* and *Lytechinus variegatus*, were obtained from the Marine Biological Laboratory, Woods Hole, Mass., and the Virginia Key area in Dade County, Fla., respectively. Eggs, obtained from *A. punctulata* by electrical shock and from *L. variegatus* by the injection of 0.45 M potassium chloride, were washed three times in filtered seawater before fixation. Three fixation methods were employed: (*a*) Paraformaldehyde-glutaraldehyde fixative of Bal et al. (1968) for 90 min followed by postfixation in 1% osmium tetroxide in 0.1 M sodium phosphate buffer, pH 7.4 for 60 min, both at 25°C. (*b*) 1% osmium tetroxide in 0.1 M sodium phosphate buffer, pH 7.4 for 1 hr at 25°C. (*c*) 2% glutaraldehyde in Millipore-filtered seawater for 1 hr at 4°C. Eggs were dehydrated in an acetone series and embedded in Araldite unless otherwise specified. Thin sections were obtained using a Porter-Blum MT-2 ultramicrotome and glass knives. Sections were stained in uranyl acetate (saturated in 25% ethanol) and lead citrate (Venable and Coggeshall, 1965). Electron microscope observations were made with Philips 100 and Philips 300 electron microscopes.

For light microscope cytochemical studies 2-$\mu$ plastic sections were placed on slides and stained with 0.1% azure A in 0.01 M sodium citrate buffer, pH 4.0. Before staining, some sections were treated with 5% periodic acid for 1 hr and then digested at 37°C with 0.5% RNase (Worthington Biochemical Corp., Freehold, N. J., ribonuclease A, code RAF, phosphate free) in 0.01 M sodium acetate buffer for 2 hr. Control sections were incubated in buffer or distilled water after the periodic acid treatment.

Thin Epon or Araldite sections of uniform thickness were used for the enzyme digestion study (Monneron and Bernhard, 1966). Sections were placed on stainless steel grids and treated with 10% periodic acid or 30% hydrogen peroxide for 30 min at 25°C if they had been fixed in osmium tetroxide. Sections were then incubated at 37°C in 0.5% RNase for 4–5 hr. Adjacent sections of the eggs used as controls were incubated in distilled water under identical conditions. Control and enzyme-treated sections were simultaneously stained as described above.

The indium trichloride staining procedure of Watson and Aldridge (1961) for nucleic acids was employed on eggs of *L. variegatus* fixed in 2% glutaraldehyde. After fixation, portions of eggs were frozen for 10 min, thawed, and incubated in either 0.01

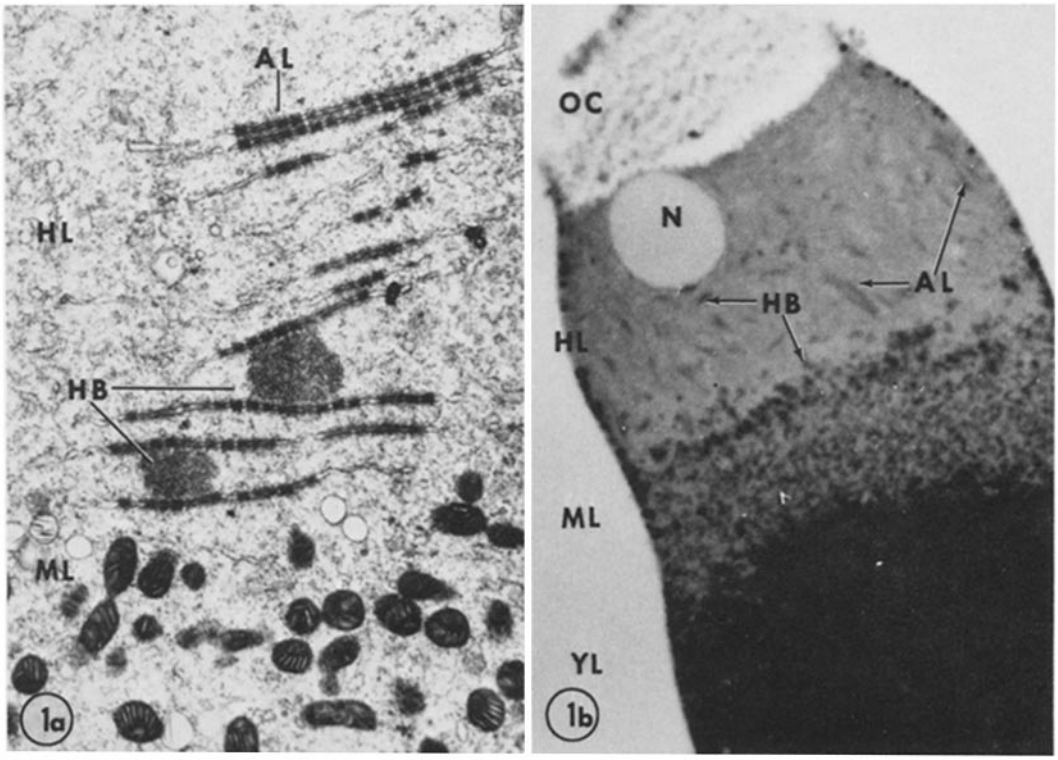


FIGURE 1 Cytochemical studies on centrifuged *Arbacia punctulata* eggs. Fixed according to Bal et al. (1968) and embedded in Araldite. (1 *a*) Electron micrograph of the hyaline (clear) and mitochondrial layers, stained with uranyl acetate and lead citrate. *HB*, heavy body; *AL*, annulate lamellae; *HL*, hyaline (clear) layer; *ML*, mitochondrial layer. ×17,100. (1 *b*) Light micrograph of centrifuged egg stained with toluidine blue. Arrows (*HB* and *AL*) indicate presence of basophilic structures in the hyaline (clear) layer. *YL*, yolk layer; *OC*, oil cap; *N* nucleus; *HL*, hyaline (clear) layer; *ML*, mitochondrial layer. ×2600.

M acetate, pH 6.0, 0.5% RNase in 0.01 M acetate buffer, or 0.2% DNase in 0.01 M acetate buffer for 3 hr at 37°C before continuation of the Watson-Aldridge procedure. As an additional control, some eggs were run through the entire procedure for staining except that no indium trichloride was present in the staining solution. Eggs were embedded in Vestopal.

## RESULTS

RNA was demonstrated in the heavy bodies of the sea urchin egg by a number of independent techniques. The first was a repetition of Afzelius's (1957) experiments using *A. punctulata* eggs instead of *Echinus esculentus* eggs. *A. punctulata* eggs were stratified by centrifugation on sucrose gradients (Tyler and Tyler, 1966). Electron microscope observations on thin sections of fixed centrifuged eggs showed that the heavy bodies and annulate lamellae were displaced to the hyaline or clear area above the mitochondrial zone (Fig. 1 *a*). This has been observed by others (Gross et al., 1960; Tilney and Marsland, 1969; Anderson, 1970). Light microscopy of thick sections of stratified eggs stained with azure A or toluidine blue showed intense basophilic structures in the hyaline layer corresponding to the position of heavy bodies and annulate lamellae (Fig. 1 *b*). Sections which were treated with ribonuclease before staining lacked these basophilic structures.

Thin sections of *L. variegatus* eggs were treated with ribonuclease using the technique of Monneron and Bernhard (1966). The granular portion of heavy bodies in sections treated with RNase

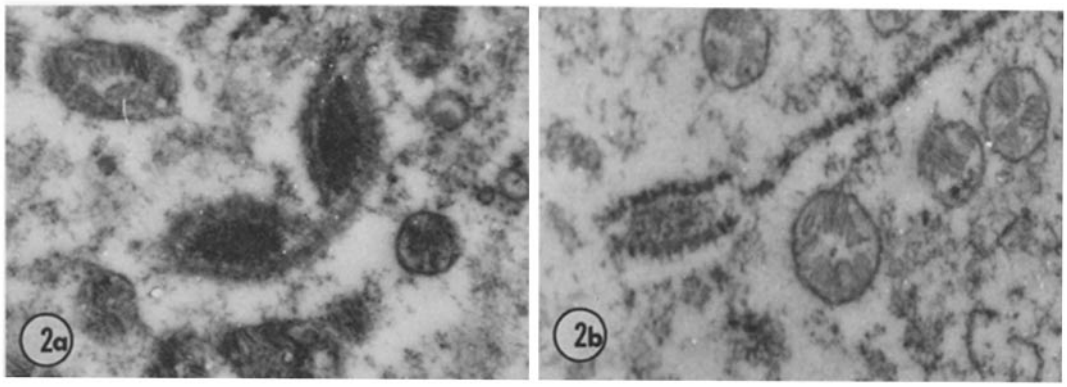


FIGURE 2 Enzyme digestion of thin adjacent sections of *Lytechinus variegatus* eggs. Fixed in $OsO_4$ and embedded in Araldite. Stained with uranyl acetate and lead citrate. ×21,000. (2 *a*) Appearance of heavy body in control section. (2 *b*) Appearance of heavy body in RNase-digested section.

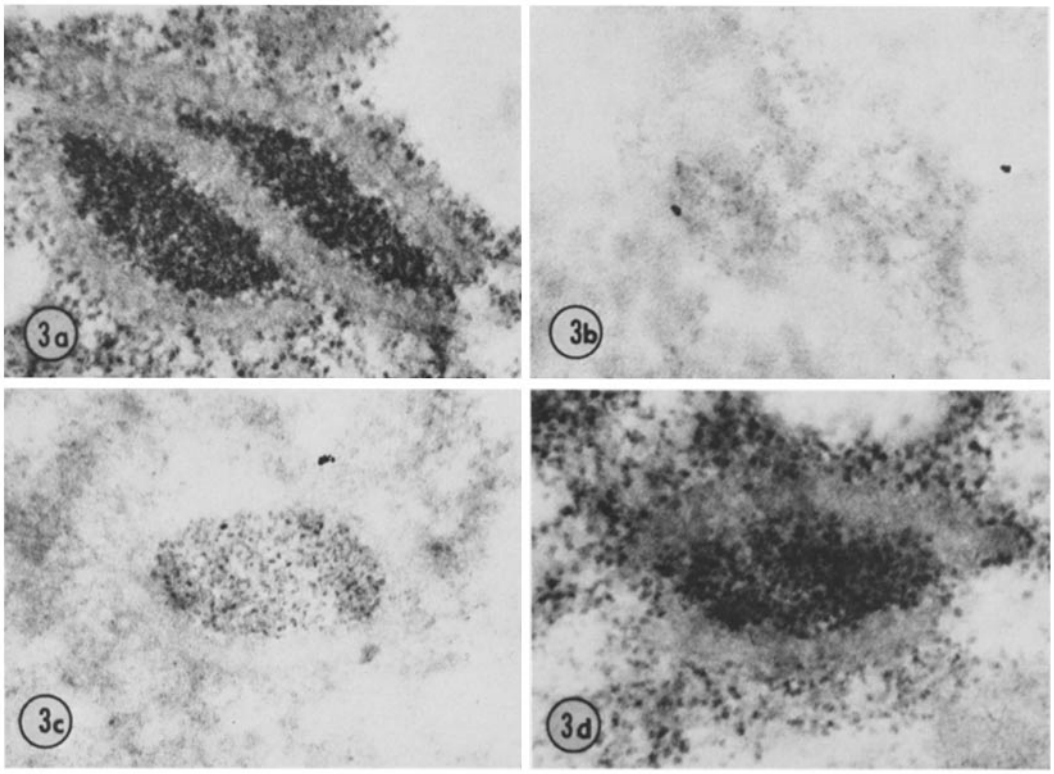


FIGURE 3 Indium trichloride stain for nucleic acids. *L. variegatus* eggs fixed in glutaraldehyde, stained with indium trichloride, and embedded in Vestopal. ×74,000. (3 *a*) Incubated and stained control. (3 *b*) Unstained control. (3 *c*) RNase treatment before staining. (3 *d*) DNase treatment before staining.

(Fig. 2 *b*) appeared less electron opaque than the controls (Fig. 2 *a*).

Nucleic acids were demonstrated in heavy bodies at the electron microscope level using the Watson and Aldridge (1961) technique for selective staining of nucleic acids with indium trichloride. The granular mass of the heavy bodies stains with indium as do the ribosomes (Fig. 3 *a*, compare with 3 *b*). Very light staining is evident in the annulate lamellae portion of the heavy

bodies. In order to decide which nucleic acid species was represented in these structures, fixed eggs were incubated with RNase or DNase before indium staining. Eggs pretreated with RNase before indium trichloride staining showed no electron-opaque, indium deposits in either the granular mass of the heavy bodies or the ribosomes (Fig. 3 *c*). The light staining of the annulate lamellae was also abolished by RNase pretreatment. Pretreatment of eggs with DNase had no apparent effect on indium staining of the heavy bodies and ribosomes (Fig. 3 *d*). However, the chromatin in eggs pretreated with DNase did not stain with indium as it did in the controls, showing that the enzyme did act within the eggs. These observations indicate that the heavy bodies of the sea urchin egg contain RNA.

## DISCUSSION

This study has shown that the heavy bodies of the sea urchin egg do contain RNA as suggested by earlier light microscope cytochemical studies (Afzelius, 1957). The RNA within the heavy bodies is located in the electron-opaque granular aggregations. In contrast to reports of RNA in the pores of the nuclear membrane of rat liver nuclei (Mentré, 1969) and of RNA-containing granules in the annuli of the nuclear membrane of amphibian oocytes (Cole, 1969), no RNA granules were detected in either the annuli of the annulate lamellae or of the nuclear membrane in the sea urchin egg. This does not rule out the possibility that a low molecular weight species of RNA is present there which might have been preserved if other fixation methods were employed (Watson and Aldridge, 1961) or that the concentration is too low for detection by the indium trichloride staining method.

Identification of the type of RNA present in the heavy bodies is not possible with the techniques employed. From the studies of Harris (1967) and Bal et al. (1968) the RNA shown to exist in heavy bodies is probably of nuclear origin. Although the staining properties of the heavy body granules are similar to those of the cytoplasmic ribosomes, other techniques must be utilized to determine whether this RNA is ribosomal, informational (mRNA), or a combination of both types possibly in the form of polysomes.

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