

DNA Content and Fragmentation of the Egg Nucleus of *Trichoplax adhaerens*

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Under culture conditions the egg nucleus of *Trichoplax adhaerens* reaches an unusually high and variable DNA content. Before the “fertilization membrane” is formed, the nucleus undergoes fragmentation. It is assumed that culture conditions differ from natural conditions by preventing the switch-over from the S-phase to the G₂-phase of the cell cycle: DNA replication continues and nuclear division is impossible. Cleavage ceases at an earlier or later stage.

Introduction

Our current knowledge about the sexual reproduction of *Trichoplax adhaerens* can be summarized as follows:

1. Under certain and reproducible circumstances female gametocytes (oocytes) are formed which grow in the intermediate cell layer. Growth is accompanied by intensive yolk production (vitellogenesis). We have described these cells which can reach a diameter of about 70–120 µm as egg cells [1]. They contain a single large nucleus of 25 µm diameter with a nucleolus (Fig. 1).

2. Under the same circumstances we found small, isolated cells in the intermediate layer which were designated as S-cells [2, 3]. We consider them as male gametocytes (spermatocytes, sperms).

3. After the growth phase, the female gametocytes can secrete a “fertilization membrane” of a specific structure. So-called cortical granules participate in its formation [2].

4. Following the formation of the “fertilization membrane” the egg cell contracts somewhat and becomes subdivided into cleavage blastomeres of about the same size.

As yet, it was not possible to observe embryonic development throughout. We have varied the culture conditions, at any rate, temperature and salinity, but were in no case successful in obtaining development beyond early cleavage stages.

Even isolated egg cells with fertilization membranes, transferred to a moist chamber, sooner or later stopped cleaving, sometimes already at the 2-cell-stage but at the latest when 32 to 64 blastomeres had been formed.

Simultaneous fixation and staining with acetocarmine indicated that the DNA content of growing egg cells must be unusually high. On the other hand, we could not unequivocally identify nuclei with the light microscope either in egg cells with a fertilization membrane or in any cleavage cells. It was therefore assumed that cleavage came to a halt because nuclear division had been disturbed from the very beginning [2].

The following microspectrophotometric (Ruthmann) and electron microscopic study (Grell and Benwitz) was conducted in order to gain more information about the nuclear situation.

Materials and Methods

The culture method and the induction of egg formation have been described previously [1, 4]. For cytophotometry of isolated cells, the tissue was first macerated in a mixture of water, glycerol and acetic acid (13:1:1 vols.) and then spun down at 500 rpm with the Shandon cytocentrifuge onto glass slides [5]. This procedure yielded isolated and flattened egg cells and enough of the presumably diploid epithelial cells for comparison. The material was fixed in methanol-formalin-acetic acid (80:10:5 ml, 80 min) and stained by the Feulgen procedure after 60 min hydrolysis in 5 N HCl at room temperature. Mea-

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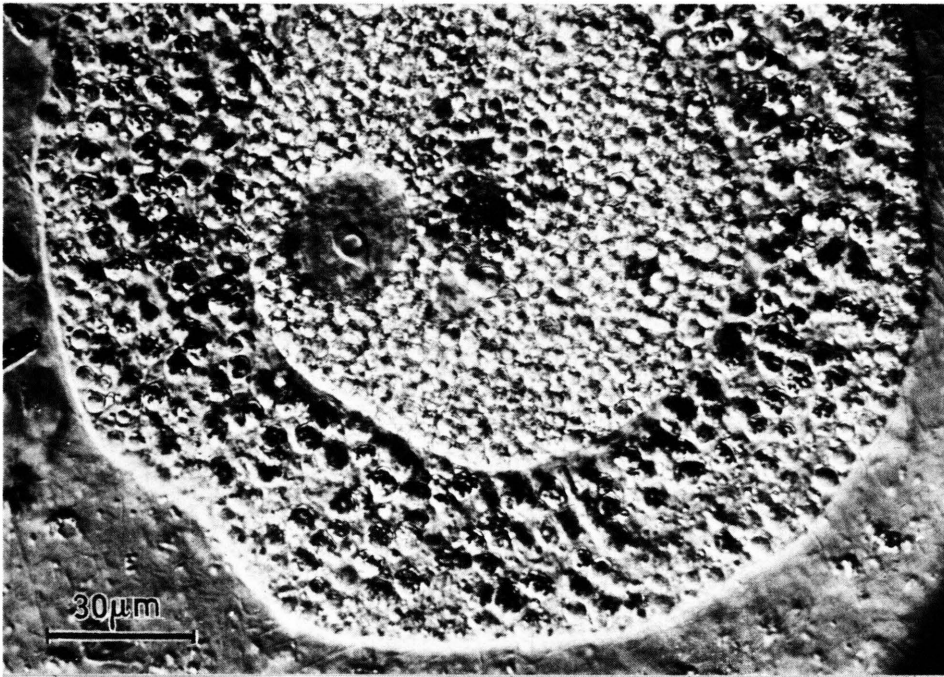


Fig. 1. Part of a living D-stage with egg cell (compressed by the cover slip). Nucleus and nucleolus clearly recognizable. Nomarski interference contrast. From Grell [1].

measurements were made at 530 nm (to avoid extinctions above 1) with the Zeiss UMSP I microspectrophotometer equipped with the rapid scanning attachment. A measuring diaphragm of 0.5 μm and a field stop of 2 μm diameter were used. Measurements were made right after mounting in "caedax"

to obtain a good refractive index match and thus minimize errors due to light scattering. On each slide enough epithelium cells (generally 15–20) were measured to obtain a reasonable mean (standard deviation < 10%). The relative DNA content of the egg cell could thus be expressed as a multiple of the DNA content of epithelium cells on the same slide.

For electron microscopy, D-phase *Trichoplax* with egg cells or cleavage stages were fixed in 1 or 2% buffered OsO_4 and embedded as described before [4].

Results and Discussion

The microspectrophotometric results are represented in Fig. 2. The relative DNA content, expressed as the ratio of the total extinction of each egg nucleus to the mean extinction of epithelial nuclei on the same slide, is plotted against the projected nuclear area. For convenience, the scales are logarithmic.

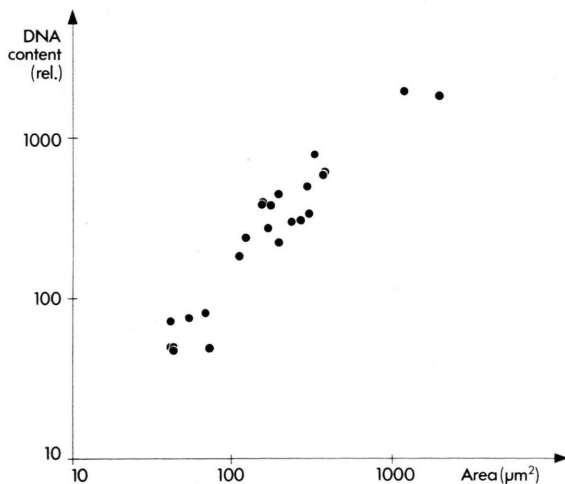
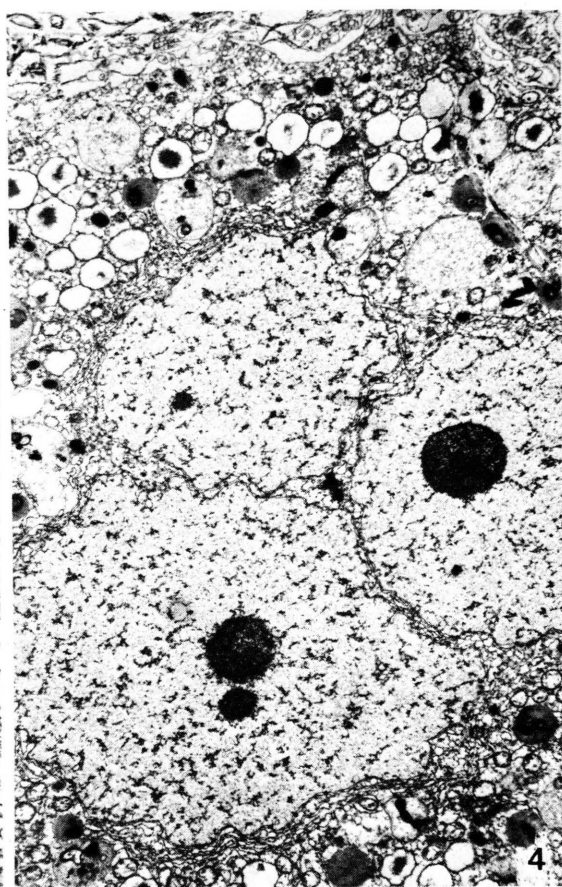
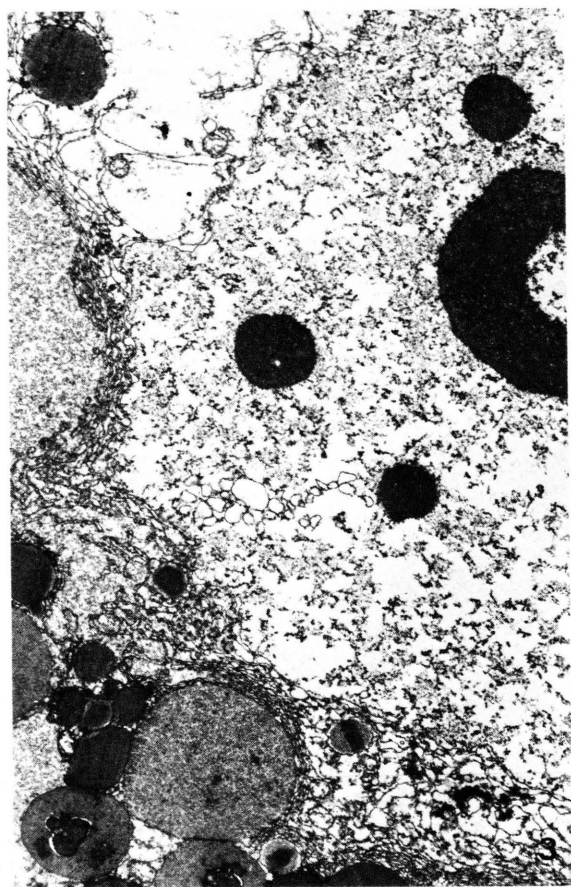
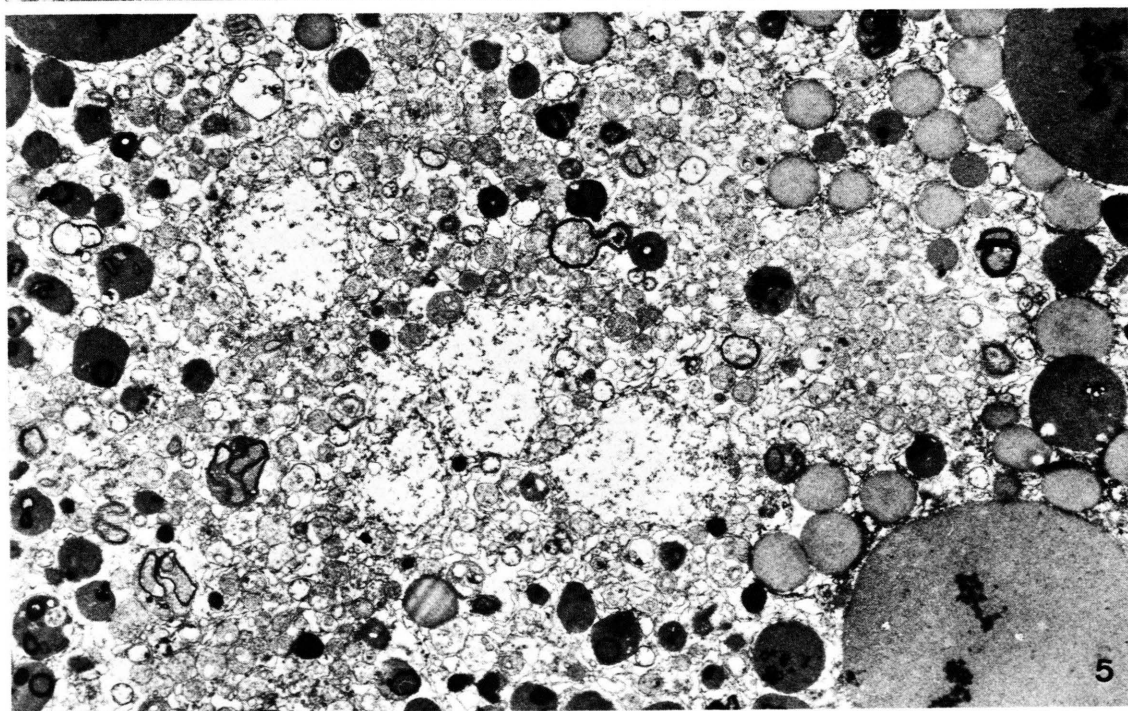


Fig. 2. DNA content of 24 egg cells (in multiples of the epithelial cell DNA content) plotted against nuclear area.

Fig. 3–5. Electron micrographs of an unfragmented egg nucleus (Fig. 3), a fragmented nucleus in an egg still without fertilization membrane (Fig. 4) and of nuclear fragments in one of the cleavage blastomeres (Fig. 5). $\times 6000$.



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There is a good correlation between DNA content and nuclear area. In a sample of 24 cells, the smallest eggs contained 48 to 50 times as much DNA as an epithelial cell, the largest one 1946 times that amount. Thus, there is a difference by a factor of 40 in DNA content, accompanied by a size range of about the same magnitude. This large range of variation can be ascribed to two causes. At least the smaller eggs not yet surrounded by a fertilization membrane must have been still growing. However, inspection of slides containing eggs with fertilization membranes also shows variations in the size of the Feulgen positive region. Thus, there is no indication that a definite final DNA content is reached.

The enormous amount of DNA in these egg cells calls for an explanation. Since DNA amplification involving loci for rRNA is not uncommon, especially in oocytes growing without nurse cells [6], this possibility should be considered in the present case. On the other hand, the immense growth of the *Trichoplax* egg cell and its nucleus might also be a laboratory effect due to a lack of physiological conditions which are normally operative in the switch-over from the S-phase to the G₂-phase of the cell cycle. If so, normal divisions would not take place and embryonic development would be bound to fail.

Electron microscopy shows only scattered nuclear fragments but no intact nuclei in blastomeres (Fig. 5). The onset of this nuclear fragmentation seems to be

correlated with the progression of the degenerative (D-) phase. In early D-phase when the animals still show lively movements, the nuclear envelope is still intact but rows of vesicles appear in the caryoplasm (Fig. 3). Later on, when locomotion has almost ceased and the animals look like small bloated spheres, the caryoplasm becomes dense and large groups of vesicles are found in the nucleus. Fig. 4 shows a fragmented nucleus in an oocyte still lacking a fertilization membrane. In fact, we have not yet found an intact nucleus in an egg which has a fertilization membrane.

Although there is considerable variation in the degree of nuclear preservation between animals in the same culture dish, our observations suggest that the oocyte and its nucleus are affected by the degenerative changes in the mother animal. Excessive DNA synthesis and growing nuclear fragility as vitality decreases might both be due to pathological changes which accompany the irreversible D-phase. On the other hand, under culture conditions, egg formation was only found after the D-phase had begun [1]. Factors which contribute to the progression into the D-phase are certainly crowding and food exhaustion. In nature, sexual reproduction might take place in intervals and depend upon a subtle interplay of various factors, possibly including seasonal variations in light, temperature and salinity. These must not necessarily imply irreversible degenerative changes of the mother animal.

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