Local accumulation of Feulgen-positive granules in the egg cortex of *Dentalium dentale* L.

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A special feature of eggs of several species of annelids and molluscs is the formation of a polar lobe at stage-specific intervals during the cleavage phase. In the scaphopod *Dentalium*, a polar lobe develops at the first, the second and the third cleavage (Wilson, 1904). The cytoplasm set apart in the polar lobes is ultimately confined to the D blastomere and its derivative cells.

Wilson observed that after removal of the polar lobe at the first cleavage a larva develops which lacks an apical tuft and the post-trochal region. After removal of the polar lobe at the second cleavage a larva develops which lacks most of the post-trochal region but possesses an apical tuft.

A similar result to the one following the removal of the first polar lobe is obtained by removal of the vegetal one-third of an unfertilized egg. These results indicate that morphogenetic determinants present in the first polar lobe are already set aside in the uncleaved and unfertilized eggs, and that the determinants for the apical tuft are no longer present in the second polar lobe.

The study of maturation in amphibian oocytes has shown that a relationship exists between nuclear DNA and Feulgen-staining granules appearing in the cortex after breakdown of the germinal vesicle (Brächet 1965, 1967). As Brachet pointed out this cortical localization may be significant with respect to the importance of the dorsal cortex (grey crescent) in later development. Therefore, we have studied the distribution of DNA in the egg cells of *Dentalium* as far as it can be traced with the Feulgen method.

**MATERIAL AND METHODS**

The experiments were carried out in May and June 1968, at the Zoological Station in Naples with eggs of *Dentalium dentale* L. The animals were kept in running sea water on a layer of sand. From the beginning of June spontaneously released eggs were obtained daily from animals brought in from the bay as well as from animals kept in the laboratory. Immediately after deposition the eggs

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are ellipsoidal but within 4–6 min they become spherical and can be fertilized. Eggs may be obtained artificially by breaking the shell and opening the ovaries. These eggs, however, take 15–20 min before they become rounded. The eggs were cultured at 22 °C in Boveri dishes in filtered and boiled sea water. Fixations were made at 5 min intervals between oviposition and third cleavage. For cytological and histochemical observations the eggs were fixed in Zenker’s fluid, sectioned, and stained either with Heidenhain’s haematoxylin or with methyl green-pyronin.

For the detection of DNA, whole eggs were fixed and stained with a Feulgen procedure. The eggs were fixed in a mixture of 80 % ethanol, 40 % formalin and acetic acid (85 cm³ + 5 cm³ + 10 cm³) and after 60 min transferred to 96 % ethanol. After hydrolysis in 5 N-HCl at 25 °C for 30 min, the eggs were stained with Feulgen’s reagent for 60 min and mounted. Control eggs were treated for 12 and 24 h with DN-ase (Worthington, 0-2 mg/ml) at 37 °C in tris buffer pH 7-4, to which 0-003 M-MgSO₄ was added. Together with each DN-ase treatment a group of eggs of a comparable stage was treated with Tris buffer pH 7-4 at the same temperature and for the same period.

RESULTS AND OBSERVATIONS

1. Maturation of unfertilized eggs

(a) Eggs obtained by artificial means. Right after deposition the eggs are ellipsoidal and contain a large centrally placed germinal vesicle with a large nucleolus (30–50 μ in diameter). At this stage ten chromosomes in the stage of diakinesis are visible (Fig. 1 A, B). The nucleolus (Fig. 2) consists of a pyro-

EXPLANATION OF FIGURES

1. Vegetal pole
2. Nuclei of follicle cells.
3. Chromosomes.
4. Germinal vesicle.
5. Nucleolus.
6. Aster.
7. Hair-like protrusions at vegetal pole.
8. Animal pole

Fig. 1 A, B. Feulgen staining of whole egg, × 560. Egg artificially released, seen from the vegetal pole. The nuclei of the follicle cells and the chromosomes of the germinal vesicle are stained. No Feulgen-positive granules at the vegetal pole.

Fig. 2. Meridional section, methyl green-pyronin, × 560. The same stage as Fig. 1. Egg obtained by artificial means, ellipsoidal; germinal vesicle intact, nucleolus with pyroninophilic peripheral part and unstained centre, follicle cells at the vegetal pole.

Fig. 3. Oblique section, methyl green-pyronin, × 560. Egg 20 min after artificial release. The nuclear membrane has disappeared, the nucleolus is disintegrating, the chromosomes are visible in the cytoplasm.

Fig. 4. Meridional section, iron haematoxylin, × 900. Hair-like protrusions at the vegetal pole.
Feulgen-positive granules in egg cortex
Feulgen-positive granules in egg cortex

ninophilic peripheral part, rich in RNA, which may contain several ‘drops’ or vacuoles and an unstained centre. The eggs are covered at the vegetal side by an annular layer of follicle cells (Figs. 1 and 2). At about 10 to 15 min after release of the eggs, the germinal vesicle opens and the nuclear membrane disappears. The nucleoplasm, the chromosomes and the nucleolus mix with the cytoplasm (Fig. 3). The nucleolus still may consist of a pyroninophilic peripheral part and an unstained centre. Fifteen to twenty minutes after release of the eggs, the follicle cells are stripped off. At the same time the eggs become spherical and are surrounded by a jelly coat. The chromosomes move towards the animal pole, together with the maturation spindle which has become visible in the meantime (Fig. 3). The nucleolus is still visible in the cytoplasm, but has started to disintegrate.

(b) Eggs released spontaneously. The eggs are ellipsoidal upon deposition, with the animal and vegetal poles located at the ends of the short axis. At the vegetal side a radial pattern is observed in which three or four dark bands of granules alternate with light bands (Fig. 5). Usually a germinal vesicle is no longer present and the follicle cells have already been stripped off. The chromosomes are condensed and are situated close to each other near the animal pole. The nucleolus is still present in the cytoplasm, but has started to disintegrate. Within 4–6 min after deposition the eggs become spherical.

2. Maturation divisions and cleavage

The first polar body is formed within 30 min after fertilization, the second polar body 30 min later. After another 30 min first cleavage starts. Second and third cleavage follow also after intervals of 30 min. The first polar lobe is formed at the first cleavage, the second and third polar lobes are formed at the second and third cleavages. At the vegetal pole hair-like protrusions are observed from oviposition until after the second maturation division (Fig. 4). During the maturation divisions a cytoplasmic protrusion is formed at this point (Fig. 7).

Figs. 5–10. Feulgen staining of whole eggs, spontaneously deposited. × 560.
Fig. 5. Egg immediately after deposition, seen from the vegetal pole. Three ‘granular’ bands alternate with three ‘light’ bands, Feulgen-positive area at the vegetal pole.
Fig. 6. Egg 6 min after deposition, seen from the vegetal pole. The egg is rounded off; Feulgen-positive granules at the vegetal pole.
Fig. 7. Egg 25 min. after deposition, shortly before first maturation division. Small cytoplasmic protrusion at the vegetal pole containing Feulgen-positive area, chromosomes situated near the animal pole.
Fig. 8. Egg 55 min after deposition, anaphase of second maturation division. Feulgen-positive area at the vegetal pole.
Fig. 9. Egg at trefoil stage, 90 min after fertilization. The first polar lobe is formed containing the Feulgen-positive area at the vegetal pole.
Fig. 10. Egg at metaphase of the second cell cycle, 20 min after the first cleavage. Feulgen-positive area at the vegetal side of the CD-blastomere.
3. Feulgen-positive granules

With the Feulgen method a positive reaction is obtained in the cortical region at the vegetal pole of the egg (Fig. 5–8). The reaction is located in the area where hair-like protrusions are found (Fig. 7). Also larger, irregular Feulgen-staining granules are found particularly in the cytoplasm of the vegetal part of the egg. Unlike the granules in the cortical region, however, these larger granules can also be stained with Schiff’s solution when hydrolysis is omitted. This shows that the staining of these granules is due to free aldehyde groups.

In eggs released spontaneously the Feulgen-positive area at the vegetal pole is found immediately after oviposition when the eggs are still ellipsoidal (Fig. 5). In eggs obtained artificially a clear Feulgen-positive area is visible after they have rounded off. While these eggs are still flattened small faintly staining granules may be seen at the vegetal pole. However, these granules are also stained when hydrolysis is omitted.

The Feulgen reaction at the vegetal pole is most pronounced during the period of the maturation divisions (Figs. 7 and 8). During this period the reaction is located in granules at the base of the hair-like protrusions and diffusely in these protrusions.

During the first and second cleavage the Feulgen-positive area is found at the vegetal side of the polar lobes (Fig. 9). Between these two cleavages the area is found in the CD cell (Fig. 10). After the second cleavage a positive reaction was no longer observed. After a DN-ase treatment the cortical layer at the vegetal pole in freshly laid eggs and cleaving eggs did not show a positive Feulgen reaction. The nuclei and the polar bodies remained unstained as well.

DISCUSSION

The results show that a Feulgen-positive area is located in the cortical region at the vegetal pole of eggs of Dentalium indicating the presence of DNA. The area is present in spontaneously released eggs immediately after deposition. However, in eggs obtained by artificial means the Feulgen-positive area cannot be demonstrated until 15–20 min after release of the eggs from the ovaries. In both cases, however, the reaction is observed at the same developmental stage, viz. immediately after the breakdown of the germinal vesicle and the rounding off of the oocytes. Apparently, the Feulgen-positive granules appear at the vegetal pole in the first phases of maturation of the oocytes.

As for the origin of the Feulgen-positive granules, several alternatives can be considered. It is possible that DNA-containing granules are deposited at the vegetal pole during oogenesis and that they are unmasked during maturation, when the nucleoplasm mixes with the cytoplasm. Another possibility is to relate their origin to the germinal vesicle, as has been done for Feulgen-positive
Feulgen-positive granules in egg cortex

granules during maturation in starfish and amphibian oocytes (Brachet, 1965, 1967; Brachet & Steinert, 1967). In this context it is interesting to note that the nucleolus still exists after the breakdown of the germinal vesicle although it has started to disintegrate (Fig. 3). Brachet (1967) and Brachet & Steinert (1967) obtained evidence that the Feulgen-positive bodies in the cytoplasm of eggs of *Xenopus* and *Asterias* are derived from the nucleoli. If that is the case in *Dentalium*, the material released from the germinal vesicle must be rapidly transported to the vegetal pole and exclusively held at this site.

The extranuclear DNA located at the vegetal pole is clearly visible up to the second cleavage. Beyond this stage the granules were not observed any more. However, the available information does not permit a definite statement that the granules are no longer present after second cleavage.

With respect to a possible role in morphogenesis the Feulgen-positive granules at the vegetal pole may be significant if we draw a parallel (1) with the Feulgen-positive bodies arising in amphibian oocytes (Brachet, 1965, 1967) at the same developmental stages and (2) with the general view about the significance of the cortex of egg cells in development (Raven, 1967). In the case of amphibian oocytes Brachet could show that Feulgen-positive granules arising from the germinal vesicle collect underneath the dorsal cortex. He related this to the importance of the dorsally located grey crescent in amphibian development. In *Dentalium* it is of interest that removal of the vegetal one-third of the unfertilized egg causes developmental abnormalities. Trochophores develop in which the post-trochal region is lacking and in which an apical tuft does not develop. Similar results are obtained when the first polar lobe is removed at first cleavage (Wilson, 1904; Verdonk, 1968).

Recent experiments (Geilenkirchen, Verdonk & Timmermans, 1970) have shown that removal of 60% of the first polar lobe at the vegetal side does not suppress the development of the apical tuft. In these experiments the Feulgen-positive granules must have been eliminated. This suggests that, if these granules are to be related with the morphogenesis of the apical tuft, their primary influence must have been exerted before first cleavage.

**SUMMARY**

1. DNA-containing granules are located in the cortical region of the vegetal pole of eggs of *Dentalium dentale* L.
2. The granules are present immediately after breakdown of the germinal vesicle and are clearly visible up to the second cleavage.
3. The granules are not observed before the breakdown of the germinal vesicle; their origin is discussed.
4. It is assumed that these granules are related to morphogenetic factors necessary for development, particularly for the development of the apical tuft and the post-trochal region.
RÉSUMÉ

Accumulation locale de granules Feulgen-positifs dans le cortex ovulaire de Dentalium dentale L.

1. Des granules contenant de l'ADN sont localisés dans la région corticale du pôle végétatif de Dentalium dentale L.

2. Les granules sont présents immédiatement après le flétrissement de la vésicule germinale et sont nettement visibles jusqu'à la seconde segmentation.

3. Les granules ne se voient pas avant le flétrissement de la vésicule germinale; leur origine est discutée.

4. On conclut que ces granules jouent un rôle parmi les facteurs nécessaires au développement, particulièrement pour celui de la touffe apicale et celui de la région post-trochale.

This work has been supported by a travel grant from the Netherlands Ministry of Education and Sciences.

REFERENCES


(Manuscript received 8 April 1969)