

3.2 Polyspermy generates Morphogenesis-defective Embryos in the Ascidian, *Ciona intestinalis* (Ascidacea, Tunicata)

Brief Note

Polyspermy generates Morphogenesis-defective Embryos in the Ascidian, *Ciona intestinalis* (Ascidacea, Tunicata)

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Abstract

Polyspermic eggs of *Ciona intestinalis* differentiate into morphogenesis-defective embryos which are a multinuclear syncytium. These morphogenesis-defective embryos hatch by dissolving their chorion at the same time control larvae hatch. The production and secretion of hatching enzyme can be separated from the process of morphogenesis. This system may be useful for understanding the mechanism of cytodifferentiation including developmental clock.

Introduction

Fertilized eggs cleave and develop in a stereotypical manner for each animal species. During development, morphogenesis and cytodifferentiation are generally closely correlated with each other; the regions of the embryo where these events occur depend on cytoplasmic and positional information. Separation of cytodifferentiation from morphogenesis may be useful for analyzing the mechanism of cytodifferentiation. This brief note reports that polyspermic eggs of *Ciona intestinalis* differentiate into morphogenesis-defective embryos, which produce and secrete hatching enzyme at the same time as control embryos.

Materials and Methods

Ciona intestinalis were collected on the coast near the Port of Nagoya, Aichi-prefecture, Japan. Eggs were obtained by puncture of the oviduct. These eggs were divided into three groups; for normal fertilization, for polyspermic fertilization and for its control fertilization.

Sperm were obtained by puncture of the sperm duct of a different individual. The sperm concentration used for insemination was calculated with a haemocytometer.

For normal fertilization and development, eggs were fertilized with 106 sperm/ml, a concentration that can give 100% monospermy. For polyspermic fertilization and development, eggs were preincubated in caffeine (5mM) seawater for 5 min and fertilized with a fairly high concentration of sperm (108 sperm/ml). As described previously, 100% of eggs are polyspermic (Fukumoto, 1990). For control fertilization and development, eggs were preincubated with caffeine (5mM) seawater for 5 min and then inseminated with 106 sperm/ml, a concentration that can give 100% monospermy. These normal, polyspermic and control eggs were cultured in filtered sea water at 15 C.

For electron microscopy, the embryos were fixed by 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at room temperature for 1 hour, followed by 2% OsO₄ postfixation in cold for one hour. The specimens were processed for TEM according to a method described by Fukumoto (1981). Observations were made on a Hitachi H-7100 TEM operated at 75 kV.

Results and Discussion

The egg of *Ciona intestinalis* is enclosed by a relatively thick and tough chorion (vitelline coat) (Fig. 1 A) to which spermatozoa bind as a prerequisite for fertilization. The chorion (vitelline coat) is externally decorated by a single layer of highly vacuolated follicle cells (Fig. 1 A) which are thought to be involved in sperm attraction, in egg floatation and in sperm penetration through the chorion (vitelline coat). Test cells (Fig. 1 E and F) are located in the perivitelline space between the egg surface and the chorion (vitelline coat). The role of the test cells in fertilization and development still remains enigmatic.

Normally fertilized eggs and fertilized control eggs become 2 cell stage embryos (Fig. 1A) at about one hour and tadpole larvae (Fig. 1 B and C) about 24 hours after fertilization. Polyspermic eggs form a monster which is typical for polyspermic fertilization (Fig. 1 D) at the same time as the 2 cell stage of control embryos. These eggs do not show any cytokinesis and morphogenesis. However, these morphogenesis-defective embryos hatch out by dissolving their chorion (Fig. 1 E and F) at the same time the control larvae hatch out. Control embryos (tadpole larvae) are made up of typical cells with various organelles (Fig. 2 A and B). Morphogenesis-defective embryos are a multinuclear syncytium. The nuclei in morphogenesis-defective embryo are relatively large and irregular in shape. The rough endoplasmic reticulum is highly developed in the cytoplasm where nuclei are located (Fig. 2 C and D).

Knaben (1936) suggested that test cells were responsible for the production and secretion of hatching enzyme. However, Lubbering and Hofman (1995) showed that the embryo itself, not test cells, secreted the hatching enzyme in *Asciella aspersa*. Morphogenesis-defective embryos and tadpole larvae derived from both normal and control eggs probably secrete hatching enzyme. The enzyme appears to be trypsin-like (Hoshi and Numakunai, 1987; Lubbering and Hofmann, 1995).

Morphogenesis-defective embryos are able to produce hatching enzyme at the same time as normal and control embryos. This experimental system may be useful for analyzing the mechanism of cytodifferentiation and developmental clock.

Explanation of Figures

Figure 1. (A)-(C): control egg and embryo; (D)-(F): polyspermic egg and embryo. (A), 2 cell stage about one hour after fertilization; (B), Tadpole larva in the chorion about 24 hours after fertilization; (C), Tadpole larva just hatching at about 24 hours after fertilization; (D), Polyspermic egg corresponding to the 2 cell stage of the control; (E), Morphogenesis-defective embryo which is hatching; (F), Hatched morphogenesis-defective embryo. Ch, chorion; Fc, follicle cells; Tc, test cells. Bar: 100um in (A) (This is also applicable to B, D, E and F); 200um in (C).

Figure 2. (A), Thick section of a control embryo at a tadpole stage; (B), Enlargement of a square region of (A). The embryo consists of cells with a typical nucleus; (C), Thick section of morphogenesis-defective embryo just after hatching; (D), Enlargement of a square region in (C). In spite of a lot of nuclei, cell boundaries are not present. Nuclei are irregular in shape and relatively large. The rough endoplasmic reticulum is highly developed. Tc, test cells; Yg, yolk granules. Bar: 100um in (A) and (C); 10um in (B) and (D).

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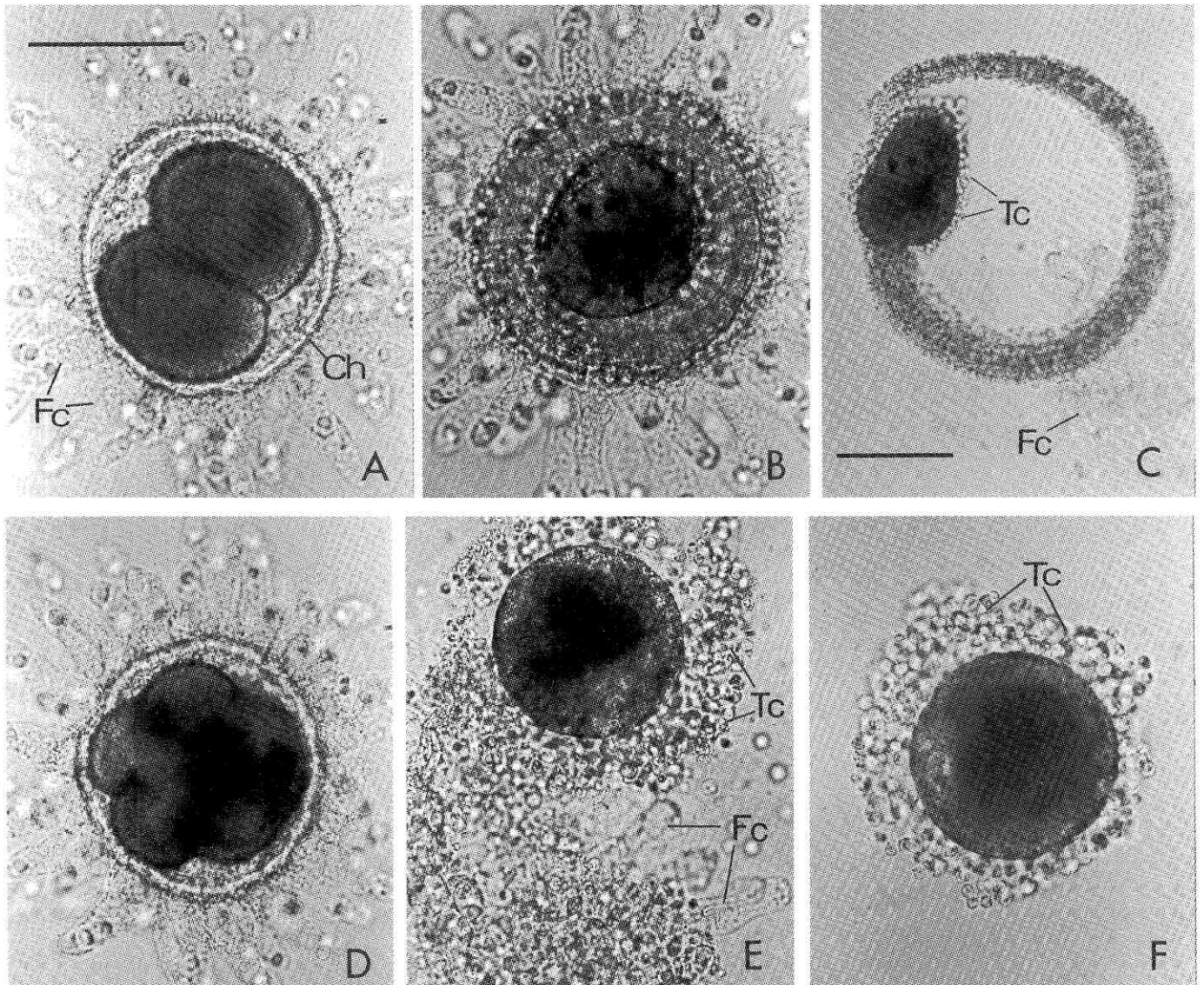


Fig.1

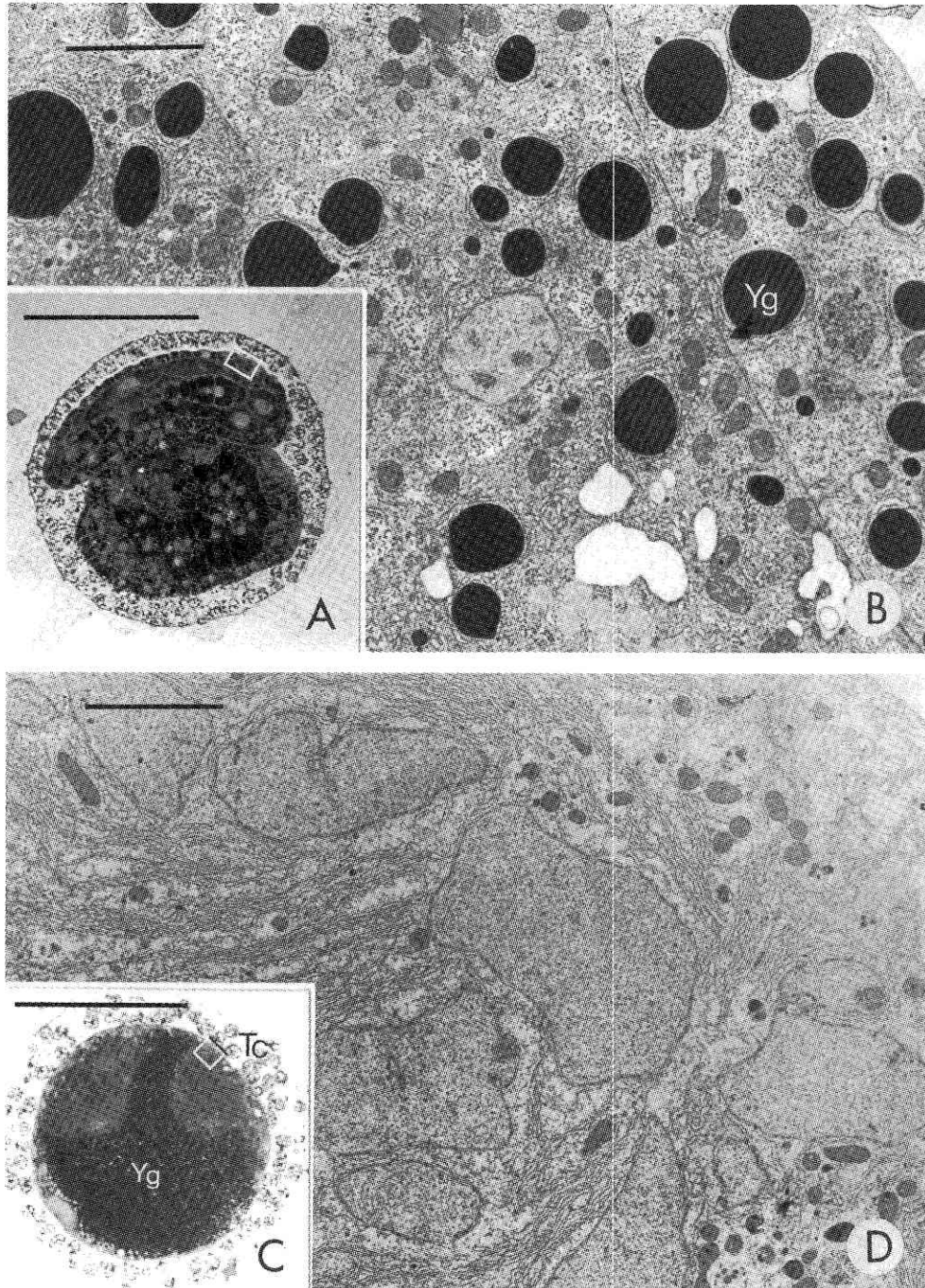


Fig.2