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Source: Zoological Science, 16(2) : 341-344

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.16.341

ZOOLOGICAL SCIENCE **16**: 341–344 (1999)

Cell Numbers in the Gut of the Embryo of the Sea Urchin *Hemicentrotus pulcherrimus*

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ABSTRACT—The number of cells in the gut of a sea urchin embryo was counted to clarify the cellular mechanisms of gut formation during development. It has been determined that in the gut of a normal embryo, the number of cells amounted to at least 49, 100, 181, 175 and 169 at the early gastrula, mid-gastrula, late gastrula, prism and pluteus stage, respectively. The percentage of the number of cells in the gut to the total number of the cells in the whole embryo was the highest at the late gastrula stage.

INTRODUCTION

It seems that the number of cells per embryo is a typical indicator of cell proliferation during development. The number of cells in the sea urchin embryo after hatching until the pluteus stage has been reported by several authors who have counted the total number of cells of the whole embryo (Driesh, 1900; Muller *et al*., 1970; Takahashi and Okazaki, 1979; Spieth and Whiteley, 1980; Stephens *et al*., 1986; Nislow and Merrill, 1988). They reported that the number of cells per embryo increased during gastrulation. Takahashi and Okazaki (1979) also reported that a half and a quarter of the larvae contained roughly a half and quarter, respectively of the number of cells of the whole embryo.

Also, many researchers have discussed the cellular mechanism of gastrulation and archenteron formation. These reports include the following: 1) that the two stages of the invagination of the archenteron are the primary invagination, during which the vegetal plate bends inward to form a short archenteron and the secondary invagination, during which the archenteron elongates across the blastocoel (Gustafson and Kinnander, 1956), 2) that gastrulation is accompanied by the rearrangement of invaginating epithelial cells (Ettensohn, 1985; Hardin, 1987), 3) that cell recognition changes accompany the ingression of primary mesenchyme cells (Fink and McClay, 1985),and 4) that archenteron elongation is a microtuble-independent process (Hardin and Cheng, 1986).

Although, Hardin (1989) estimated the total number of cells in the archenteron during secondary invagination in *Lytechinus pictus*, on the basis of scanning electron micrographs and reported that the cell number in the archenteron does not change during the invagination. The number of cells in the gut during development is still unknown. The number of cells in the gut seems to be one of the basic data for the study of the cellular mechanism of gastrulation and archenteron formation.

In the present study, the number of cells in the gut of the sea urchin embryo during archenteron formation was counted to provide the study of the cellular mechanism of archenteron formation with special reference to cell proliferation.

MATERIALS AND METHODS

Sea Urchin embryo

Gametes were obtained from the sea urchin, *Hemicentrotus pulcherrimus*, by an injection of 0.5 M KCl into the body cavity. The eggs were washed twice with filtered sea water(FSW) and were inseminated. They were then washed three times with FSW to eliminate excess sperm and allowed to develop at 20°C with gentle stirring. The embryos kept at 20°C were collected by a hand-driven centrifuge at early gastrula (21 hr after fertilization), mid-gastrula (24 hr after fertilization), late gastrula (28 hr after fertilization), prism (32 hr after fertilization), and pluteus (48 hr after fertilization) stage, respectively. The procedure of isolation of archenteron from the embryo was as described previously (Mizoguchi *et al*., 1989).

Cell number counting

At each stage mentioned above, the whole embryo or isolated archenterons were collected by a hand-driven centrifuge. A tiny drop of the embryo or gut suspension was put on a glass slide and about 2 times as much aceto-orcein as the volume of the suspension was added in order to fix the embryo and the gut and stain the nuclei simultaneously (Takahashi and Okazaki, 1979). After 2 hr, the embryos and guts were compressed between the cover and the glass slide until the constituent cells were spread into a monolayer (Takahashi and Okazaki, 1979). The preparation of aceto-orison was done according to the method used by Takahashi and Okazaki(1979). The numbers of the nuclei of the compressed embryos and guts were counted on photographs.

Immunohistochemistry

Exposure of the embryos to 0.1 mM of 5-bromodeoxyuridine (BrdU) for 10 min at 20°C was performed at the stages mentioned above. BrdU-labeled embryo cells were considered to be those having incorporated BrdU into the nuclear DNA. To detect BrdU-labeled embryo cells, immunohistochemistry using an anti-BrdU monoclonal

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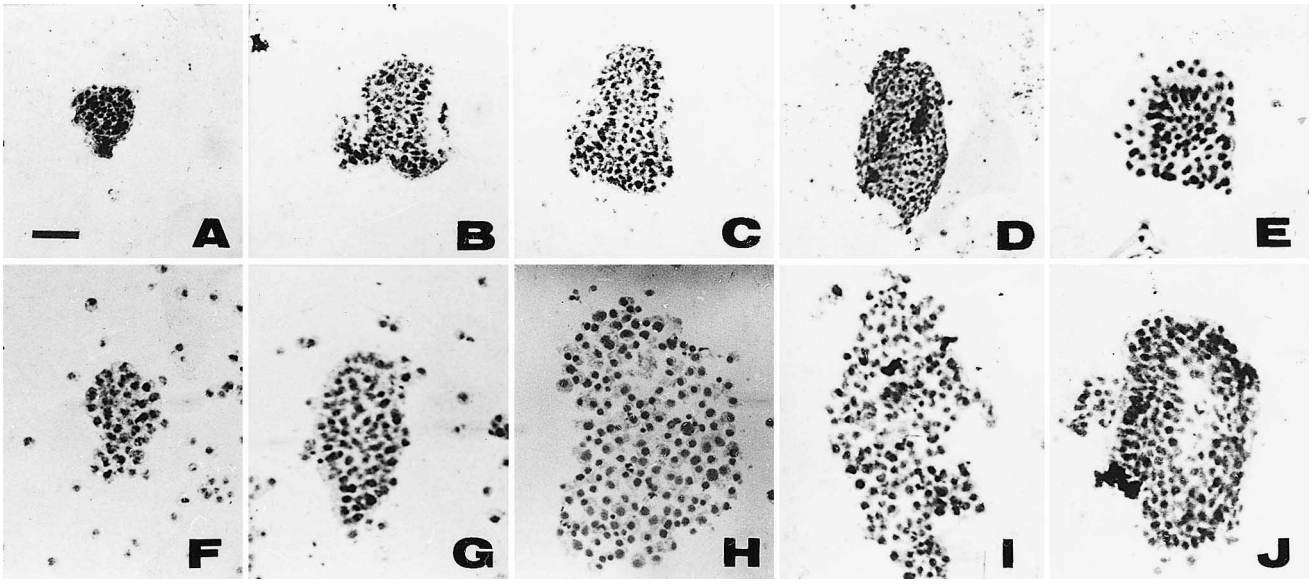

Fig. 1. Photographs of the isolated guts fixed and stained with aceto-orcein. The isolation of the guts from the embryo was performed. The guts from the embryo **A**: at the early gastrula stage (21 hr after fertilization at 20°C), **B**: at the mid-gastrula stage (24 hr after fertilization at 20°C), **C**: at the late gastrula stage (28 hr after fertilization at 20°C), **D**: the prism stage (32 hr after fertilization at 20°C) and **E**: the pluteus stage (48 hr after fertilization at 20°C). The isolated gut stained with aceto-orcein and squashed. **F**: at the early gastrula, **G**: at the mid-gastrula, **H**: at the late gastrula, **I**: at the prism and **J**: at the pluteus stage, respectively. The bar indicates 25 μm.

antibody (Becton-Dickinson Monoclonal Centre, Inc.) was conducted on paraffin sections. Deparaffinized sections were treated with ribonuclease A (Sigma) dissolved in PBS (1 mg/ml) for 30 min at 37°C. DNA denaturation was carried out following the method of Tanaka (1990). Immunohistochemical staining was carried out according to the procedure for a biotin-streptavidin system (Amersham International),using biotinylated antibody from sheep immunized against mouse IgG as the second antibody. This was followed with a light microscopy.

RESULTS

Fig.1 shows the photographs of the isolated guts fixed and stained with aceto-orcein. Shown in these figures are guts of: early gastrulae (21 hr after fertilization at 20°C, Fig. 1A) and their compressed guts (Fig. 1F); mid-gastrulae (24 hr after fertilization at 20°C, Fig. 1B) and their compressed guts (Fig. 1G); late gastrulae (28 hr after fertilization at 20°C, Fig. 1C) and their compressed guts (Fig. 1H); prisms (32 hr after fertilization at 20°C, Fig. 1D) and their compressed guts (Fig. 1I); and plutei (48 hr after fertilization at 20°C, Fig. 1E) and their compressed guts (Fig. 1J). It seems that almost all the intact guts were isolated from the embryo and were stained with aceto-orcein.

Although a small number of cells come off the embryo or gut and some of the cells overlapped other parts of the embryo or gut during the preparation of the counting or cell number, the number of cells of the compressed whole embryos or guts seems to reflect the cell number of the whole embryo or gut. The number of the cells come off the embryo or gut was excepted from the data of the cell number.

The total cell number in the whole embryo and the cell number in the isolated gut are shown in Fig. 2. The total cell

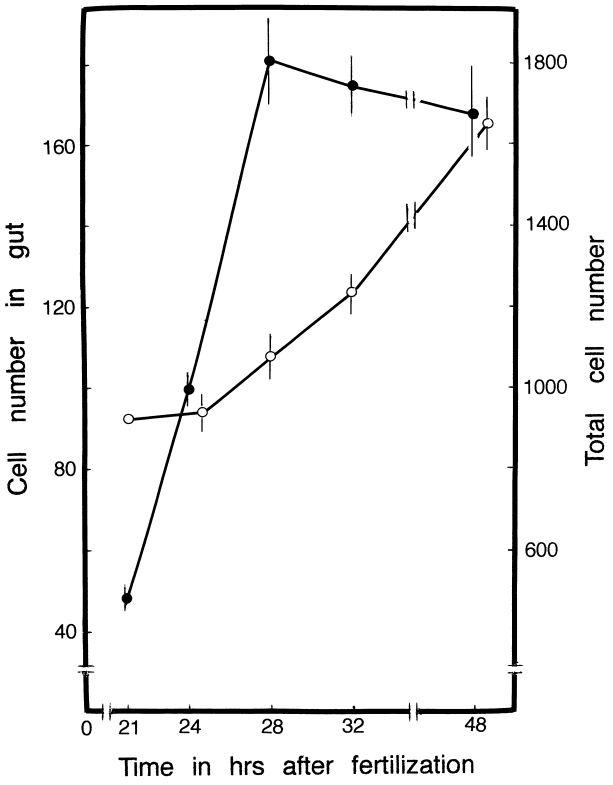

Fig.2. The total cell number of the embryo and the number of cells in the isolated gut. The cell number was counted from the embryos or isolated guts stained with aceto-orcein and compressed. Open circles (○) indicate the number of cells of the embryo(ordinate: right) and solid circles (●) indicate the number of cells in the isolated gut(ordinate left). The stages are shown in abscissa. The values shown are the mean of 30 embryos or isolated guts±SEM. The vertical bar shows SEM.

number of the embryo continued to increase exponentially during archenteron formation. The cell number in the isolated gut at the early gastrula stage was at least 49 and increased linearly until it reached at least 181 at the late gastrula stage. The number of cells in the gut of the prism and the pluteus was at least 175 and 169, respectively.

Fig. 3 shows the percentage of the number of cells in the gut to the total number of cells in the whole embryo from the early gastrula to the pluteus stage. The percentage of the number of cells in the gut to the total number of cells in the whole embryo increased linearly between the early gastrula stage and the late gastrula stage and gradually decreased between the late gastrula stage and the pluteus stage.

Fig. 4 shows distribution of BrdU incorporated cells (S-phase cells) of the early gastrula (Fig. 4A) and the mid-gastrula (Fig.4B). In both embryo, the S-phase cells was found in the structure of archenteron.

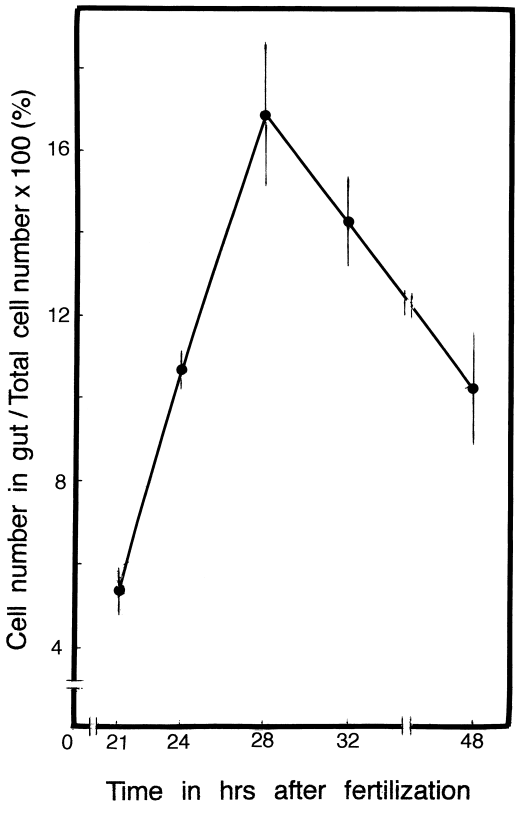

Fig. 3. The percentage of the number of cells in the gut to the total number of cells in the whole embryo. The stages are shown in abscissa. The values shown are the mean of 3 experiments±SEM. The vertical bar shows SEM. Ten embryos and ten guts were counted in each experiment. From this, the number of cells in the gut and the total number of cells in the whole embryo were calculated.

DISCUSSION

In the present study, the total cell number of the embryo continued to increase exponentially during archenteron formation. This result agrees with the results of other researchers (Driesh, 1900; Muller *et al.*, 1970; Takahashi and Okazaki 1979; Spieth and Whiteley, 1980; Stephens *et al.*, 1986; Nislow and Merrill, 1988).

A small number of the cells come off the gut of the embryo, especially early and mid-gastrulae (Fig. 1F, 1G). After the late gastrula stage, almost all the cells of the gut of the embryo were observed in the compressed gut(Fig. 1H, 1I, 1J). It seems that these results are due to the changes of cell adherence during archenteron formation (Fujisawa and Amemiya, 1985).

It seems that the rate of increase of the cell number in the gut is higher than the total cell number of the embryo during the early gastrula stage (Fig. 2). The total cell number in the whole embryo from the early gastrula stage to the mid-gastrula stage increase by about 30. On the other hand, the cell number in the isolated gut at the same period increase by about 50. The total cell number in the whole embryo from the mid-gastrula stage to the late gastrula stage increase by about 120. On the other hand, the cell number in the isolated gut at the same period increase by about 80. Hence, it seems that the cell number in the isolated gut does not depend only on cell division of the gut. The S-phase cells were found in the structure of archetneron in early gastrula and mid-gastrula (Fig. 4). S-phase thought to be a marker of cell proliferation. Hence, it seems that cell proliferation occur in the gut occurs from the early gastrula stage to the mid-gastrula stage. Although, the number of cells that participate in the archenteron structure from the embryo structure such as embryo-wall, is unknown,

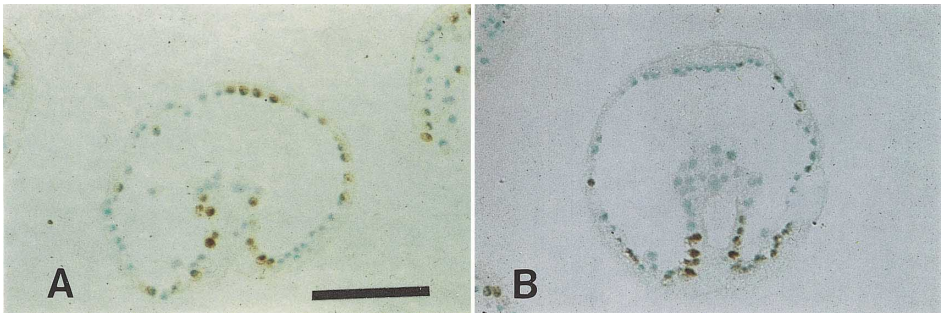

Fig. 4. Distribution of S-phase cells in the embryo. Detection of S-phase cells was performed. Labeling with lower, non-inhibitory concentration of BrdU (0.1 mM) was performed at the early gastrula stage (21 hr after fertilization at 20°C) and the mid-gastrula stage (24 hr after fertilization at 20°C). Brown spots indicate S-phase cells. **A**: early gastrula, **B**: mid-gastrula. Bar indicates 50 μm.

it is probable that some cells from the embryo structure such as embryo-wall, are involved in archenteron formation. It seems that cell proliferation of the embryo structure such as embryo-wall, is higher than that of the gut, concerning the contribution of cell proliferation of the whole embryo, especially from the mid-gastrula stage to the late gastrula stage.

On the other hand, it has been reported that the total number of cells in the archenteron does not change during secondary invagination in *L. pictus*, on the basis of cell counts made from scanning electron micrographs (Hardin, 1989).

The total number of cells in the archenteron increased from the early gastrula stage to the mid-gastrula stage observed on embryo section in *H. pulcherrium* (data not shown). Hence, in cell number of gut during secondary invagination, it may differ to some extent depending on sea urchin species.

It has been also reported that in *L. variegatus* regionalized cell division is extensive at the gastrula stage, and may contribute significantly to the process of invagination (Nislow and Morrill, 1988).

The distribution of S-phase cells was found around the blastopore (Fig. 4). This agrees with the results of Nislow and Morrill (1988). However, it seems that the number of cells of S-phase cells in the archenteron is larger in *H. pulcherrium* (Fig. 4) compared with *L. variegatus* from the mid-gastrula stage to the late gastrula stage. It may be due to the difference of sea urchin species.

In the present study, the number of cells in the isolated gut of the sea urchin embryo during archernteron formation was presented. It may contribute to the analysis of cell proliferation including cell cycle and/or cell death of sea urchin embryo during archenteron formation.

Further studies on cell proliferation during archenteron formation is now under investigation.

ACKNOWLEDGEMENT

The author wishes to thank Prof. Akiya Hino (Kanagawa University, Japan) for the culture of the sea urchin embryo and valuable suggestions. The author also thanks Prof. Bart Weyand (University of Southern Maine, USA) for his comments on the manuscript. Finally, especial thanks to Dr. Shoji Tanaka (Mitsubishi Kagaku Institute of Life Sciences, Japan) for technical advice on immunohistochemistry.

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(Received April 3, 1998 / Accepted December 10, 1998)