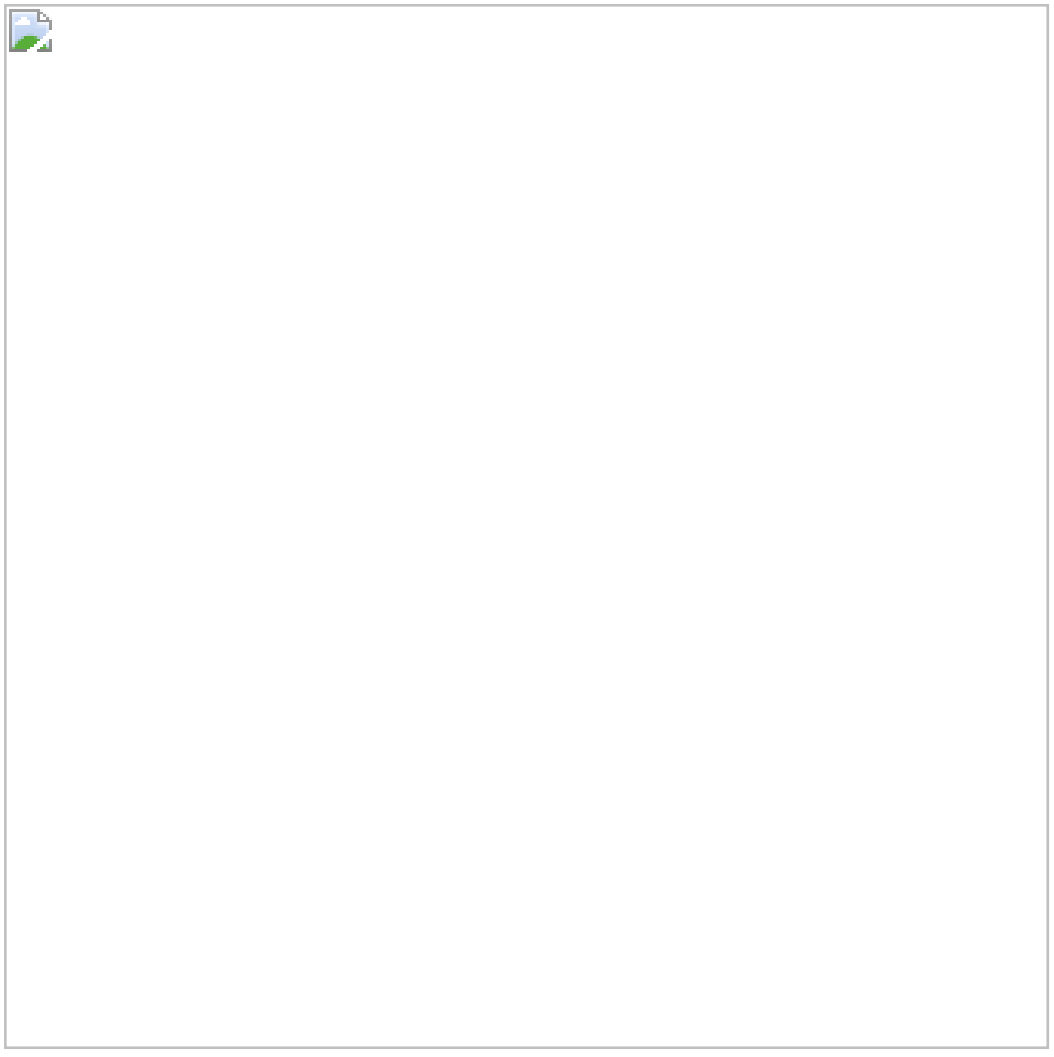

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[SHORT COMMUNICATION]

Potential of *veg*2 Blastomeres to Induce Endoderm Differentiation in Sea Urchin Embryos

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ABSTRACT—Two different modes of gastrulation in sea urchin embryos have been reported. The first mode, reported in *Hemicentrotus pulcherrimus* and some other species, consists of two phases: a primary and a secondary invagination. The second mode involves gastrulation with a continuous convolution of cells near the blastopore; this mode has been reported to occur in the embryos of the sand dollar, *Scaphechinus mirabilis*. The rudimentary gut is comprised of fewer cells in the embryos of the former species than in the latter. We assumed that the differences in gastrulation modes could be related to the different potentials of the *veg*2 layer to induce endoderm differentiation in the upper layer. In the present study, we produced chimeric embryos consisting of an animal cap recombined with *veg*2 layer blastomere(s) to compare the inductive effect of the *veg*2 layer and/or the blastomere(s) in *H. pulcherrimus* and *S. mirabilis* embryos. Our results showed that the inductive effect of the *veg*2 layer is stronger in *S. mirabilis* embryos than in *H. pulcherrimus* embryos. Moreover, it was suggested that the difference in the strength of inductive effects of *veg*2 layers is related to the difference in gastrulation modes.

Key words: echinoid development, induction, cell-cell interaction, *veg*2 blastomere, gastrulation mode

INTRODUCTION

Gastrulation in sea urchin embryos has been reported to consist of two different phases (Dan and Okazaki, 1956; Gustafson and Kinnander, 1956): primary invagination (autonomous buckling of the vegetal plate) and secondary invagination. Secondary invagination can be further divided into two steps. In the first step, the archenteron elongates via a rearrangement of the cells in the rudimentary gut that was formed by the primary invagination (Ettensohn, 1985; Hardin and Cheng, 1986). In the second step, the secondary mesenchyme cells (SMCs) raise the gut by the filopodial constriction to complete gastrulation (Hardin, 1988). In this mode of gastrulation, no new cells are added to the archenteron after the primary invagination is completed (Kominami and Masui, 1996).

Although this gastrulation process has been observed in numerous echinoid species, a second type of gastrulation has been reported in the sand dollar, *S. mirabilis*. Gastrulation in this organism does not appear to have discernible phases (Kominami and Masui, 1996; Kominami and Takata, 2000). Convolution of the cells near the blastopore persists, even during the late gastrula stage.

At the 64-cell stage of the normal development of echinoid embryos, the rudimentary archenteron consists of descendants of part of the *veg*1 layer and all of the *veg*2 layer (Ruffins and Ettensohn, 1996; Logan and McClay, 1997). Kominami and Masui (1996) suggested that more cells derived from the *veg*1 layer contribute to the completion of the archenteron in *S. mirabilis* embryos than in *H. pulcherrimus* embryos, which exhibit the typical gastrulation process. Kominami and Masui (1996) also indicated that the number of cells in rudimentary gut was much higher in *S. mirabilis* embryos than in *H. pulcherrimus* embryos.

It has been hypothesized that a cascade of signals originating in the micromeres produced at the 16-cell stage flows from the vegetal to the animal direction for later cell

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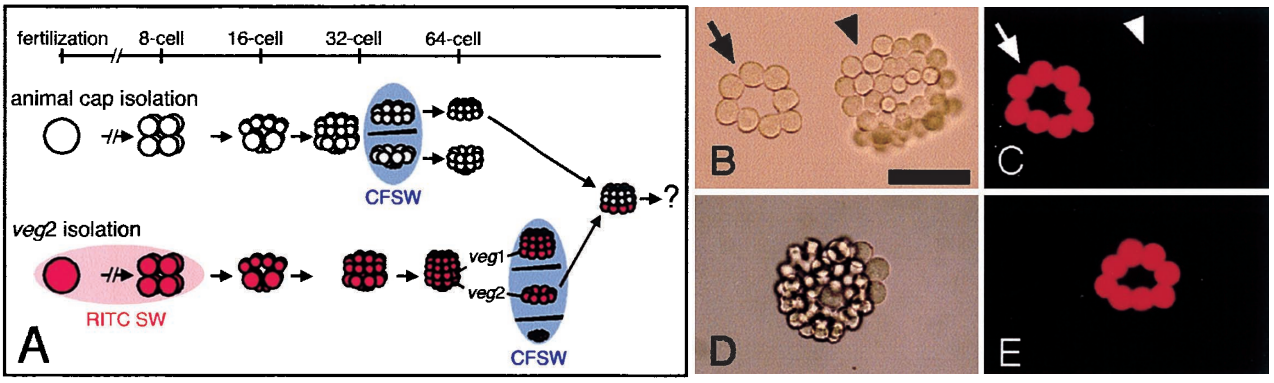

Fig. 1. Experimental procedure for producing recombinant embryos. The diagram shows the recombination of the animal cap with the *veg*2 layer (A). Bright field images (B, D) and fluorescent images (C, E) of the animal cap (arrowheads) and labeled *veg*2 layer (arrows) isolated from *S. mirabilis* embryos. Bar, 100 μm

specification (Davidson, 1989), and various experiments have supported this concept. Pluteus larvae develop from chimeric embryos consisting of micromeres and presumptive ectoderm (animal cap) (Horstadius, 1973; Amemiya, 1996; Minokawa *et al*., 1997; Minokawa and Amemiya, 1998). Both ectopic SMCs (McClay *et al*., 2000) and the secondary archenteron (Ransick and Davidson, 1993) have been found to be induced when exogenous micromeres are transplanted, indicating that the micromeres emit the inductive signal, although the timing of the micromere signal for archenteron induction might be much later than the 16-cell stage (Minokawa and Amemiya, 1999; Ishizuka *et al*., 2001). Micromere deletion at the 16-cell stage results in considerable reduction of the expression of the vegetal plate specific gene *Endo16* (Ransick and Davidson, 1995) and in no specification of SMCs (Sweet *et al*., 1999; McClay *et al*., 2000).

At the 6th cleavage, macromere descendants divide horizontally to form *veg*1 and *veg*2 layers. The inductive effect of *veg*2 cells is necessary for part of the *veg*1 cells to differentiate into endoderm (Logan and McClay, 1999). In the present study, we investigated the inductive activity of the *veg*2 layers in *H. pulcherrimus* and *S. mirabilis* embryos as a means of determining whether the difference in the activity of the inductive effects of the *veg*2 layers is connected to the different modes of invagination. Our results showed that the ability of the *veg*2 layer to induce endoderm differentiation in the animal-cap cells of *S. mirabilis* embryos is greater than in *H. pulcherrimus* embryos.

MATERIALS AND METHODS

Animals and embryos

Adult sea urchins (*Hemicentrotus pulcherrimus*) were collected around the Miura peninsula, while adult sand dollars (*Scaphechinus mirabilis*) were provided by the Otsuchi Marine Research Center and collected in the Inland Sea of Japan (Setonaikai). Gametes were obtained by the intracoelomic injection of 0.1 M acetylcholine chloride. The eggs were washed several times with artificial sea water (ASW; Jamarin-U, Jamarin Laboratory, Osaka, Japan). Fertilization was performed in 1 mM of aminotriazole (ATA) sea water to facilitate demembranization by preventing the fertilization envelopes from hardening. The fertilization envelopes were removed by pipetting the egg suspension with a fine-bore pipette. The demembranized eggs were cultured at about 14°C for *H. pulcherrimus* and at about 18°C for *S. mirabilis* in a Petri dish coated with 1.2% agar and filled with ASW.

Recombination of *veg*2 layers with animal caps

The recombinants were produced according to the basic method of Minokawa and Amemiya (1999), with some minor adjustments.

Animal caps were isolated at the 32-cell stage in calcium-free sea water (CFSW) using a glass needle from a group of embryos that had been cultured in ASW. Another group of sibling embryos was cultured in ASW containing 50 μg/ml of rhodamine isothiocyanate (RITC; R-1755. Sigma Chemical Co., St Louis, MO, USA) from the 1 to 8-cell stage and then transferred to another dish filled with ASW. The *veg*2 layers were isolated from the RITC-labeled embryos at the 64-cell stage in CFSW using a glass needle. The animal cap recombinants with the labeled *veg*2 layer were produced and cultured in another dish coated with 1.2% agar and filled with ASW containing 100 units/ml of penicillin and 50 μg/ml of streptomycin sulfate (Fig. 1).

The developing recombinants were observed using an epifluorescence microscope (Optiphot, Nikon Corporation, Tokyo, Japan).

Detection of alkaline phosphatase activity

The procedures for detecting alkaline phosphatase activity were essentially identical to those reported by Minokawa and Amemiya (1999).

RESULTS AND DISCUSSION

Animal halves of sea urchin embryos isolated during the early cleavage stage differentiate exclusively into ectoderm to form a permanent blastula. However, the animal halves also have the potential to develop into pluteus-like larvae under the inductive influence of other blastomeres that have been recombined (Horstadius, 1973; Amemiya, 1996; Logan and McClay, 1999). We produced recombinant embryos [A+V2(8)] (Fig. 1) consisting of an animal half (A) and a *veg*2 layer (V2) that contained eight blastomeres (8) to determine the inductive potential of the *veg*2 layer. We

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judged the strength of the inductive effect of *veg2* cells, by how many parts of the tripartite digestive tract originated from the animal-half cells. As the inductive effect increased in this system, cells derived from animal cap to have contributed to more parts of the tract could be assumed.

All of the [A+V2(8)] embryos in *H. pulcherrimus* developed into pluteus larvae (25/25) with a differentiated digestive tract (Fig. 2A,B). The labeled cells showed that the foregut and midgut were composed of *veg2* descendants, whereas the hindgut did not contain any labeled cells in nearly half of the recombinants (Table 1). In this half of the recombinants, the hindgut had differentiated from the animal-half cells under the inductive influence of the *veg2* cells (Fig. 2A,B, double arrowheads). In the other recombinants, however, the labeled *veg2* cells prevailed throughout the entire tripartite digestive tract, indicating that induction had not occurred in these cases (Table 1). In *S. mirabilis*, a number of [A+V2(8)] embryos also developed into pluteus larvae (22/29) with a differentiated digestive tract (Fig. 2C,D). In almost all of these recombinants, only the foregut (Fig. 2C,D, arrowheads) was composed of the *veg2*-descendant cells, with the midgut (Fig. 2C,D, arrows) and hindgut (Fig. 2C,D, out of focus) being derived from the animal-half cells (Table 1).

Table 1. The effect of *veg2* layer on the induction of endoderm differentiation in animal-half cells. Each number is shown with percentage in parentheses

	N	none	H gut	M, H gut	F, M, H gut
H. pulcherrimus	25	13 (52)	12 (48)		
S. mirabilis	22			21 (95.5)	1 (4.5)

Abbreviations; H, hind; M, mid; F, fore

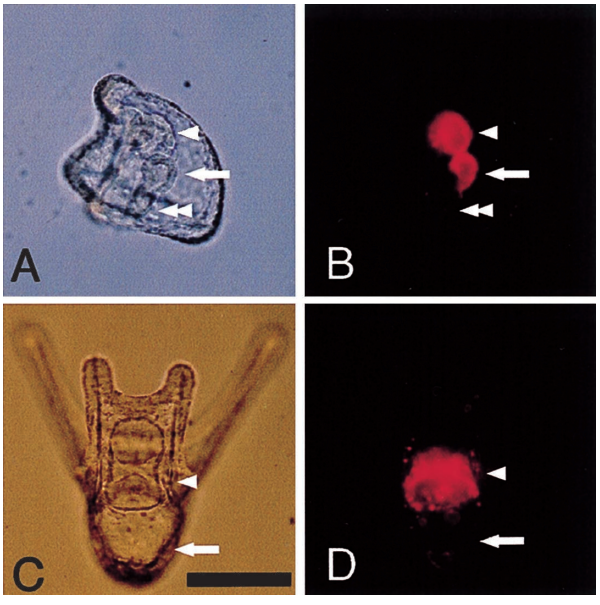

Fig. 2. Larvae with a digestive tract derived from recombinant embryos [A+V2(8)] in *H. pulcherrimus* (A, B) and *S. mirabilis* (C, D) two days after fertilization. A and C show bright field images, viewed from the left and dorsal sides, respectively. B and D show fluorescent images corresponding to A and B, respectively. Arrowheads, arrows and double arrowheads point to the foregut, midgut and hindgut, respectively. Bar, 100 μm

Alkaline phosphatase (APase) activity was examined to confirm whether the animal-half cells had functionally differentiated into a digestive tract. In both species, APase activity was expressed in the midgut and hindgut of the digestive tract derived from the animal-half cells in the recombinant larvae (Fig. 3), as in the normal larvae (Amemiya, 1996; Minokawa *et al*., 1997).

The midgut and hindgut of *S. mirabilis* were entirely derived from the animal-half cells in all [A+V2(8)] chimeric embryos that developed the archenteron. In *H. pulcherrimus*, however, less than half of the chimeric embryos exhibited animal-half cells, and only in a limited region of the hindgut. These results indicate that the inductive potency of the *veg2* layer in *S. mirabilis* is higher than in *H. pulcherrimus*. As described in the introduction, the mode of gastrulation in *S. mirabilis* embryos is different from that in *H. pulcherrimus* (Kominami and Masui, 1996). The number of cells in the archenteron in *S. mirabilis* embryos increased as gastrulation proceeded, whereas the numbers were almost constant in *H. pulcherrimus*. On the basis of the results obtained in the present study, the following appear to be possible explanations for the difference in modes of gastrulation in these two species. The inductive activity of the *veg2* layer or the competence of the animal side cells in *H. pulcherrimus* embryos is weaker than in *S. mirabilis*, so that fewer cells in the animal side layer in *H. pulcherrimus* embryos are specified to endoderm than in *S. mirabilis*. It is also possible that the inductive signal of micromeres drives that of the *veg2* layer, as reported by Davidson (1989) and Davidson *et al*. (1998). The difference in the intensity of the inductive effect of the *veg2* layers may be associated with the difference in that of the micromeres between the two species. We are now producing the interspecies chimeric embryos derived from an animal cap isolated from one species recombined with a *veg2* layer or a quartet of micromeres isolated from the other in order to investigate the above possibilities.

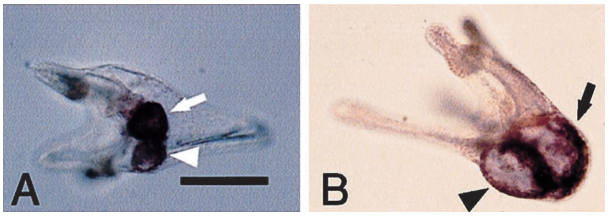

Fig. 3. Alkaline phosphatase (APase) activity in larvae derived from recombinant embryos [A+V2(8)], viewed from the left side. APase activity is detected in the induced hindgut of *H. pulcherrimus* (A) and the induced mid- and hindgut of *S. mirabilis* (B). Arrows and arrowheads point to the midgut and hindgut, respectively. Bar, 100 μm

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Next, we produced several types of chimeric embryos [A+V2(x)] consisting of an animal cap (A) recombined with four, two, and one blastomere(s) (number, x) of the *veg2* layer (V2) in *H. pulcherrimus*. More anterior fractions of the digestive tract were induced to differentiate from the animal-half cells when fewer numbers of *veg2* blastomeres were recombined (Fig. 4A), indicating that the inductive effect of the *veg2* blastomeres rose as the number of *veg2* blastomeres decreased. Chimeric *S. mirabilis* embryos [A+V2(1)] consisting of an animal cap (A) recombined with just one (1) of the *veg2* (V2) blastomeres were produced, and the inductive potential of the *veg2* blastomere was found to be higher than that of the *veg2* layer (Fig. 4B). These results show that the inductive activity of *veg2* blastomere(s) increased as the number of *veg2* blastomeres decreased. The mechanism(s) responsible for the decreased number of *veg2* blastomeres exerting stronger inductive activity than the increased number of blastomeres is still unknown. One possibility is that a lateral interaction between *veg2* blastomeres inhibits the inductive activity of neighboring cells, as reported by Henry *et al*. (1989) in regard to the mesomeres.

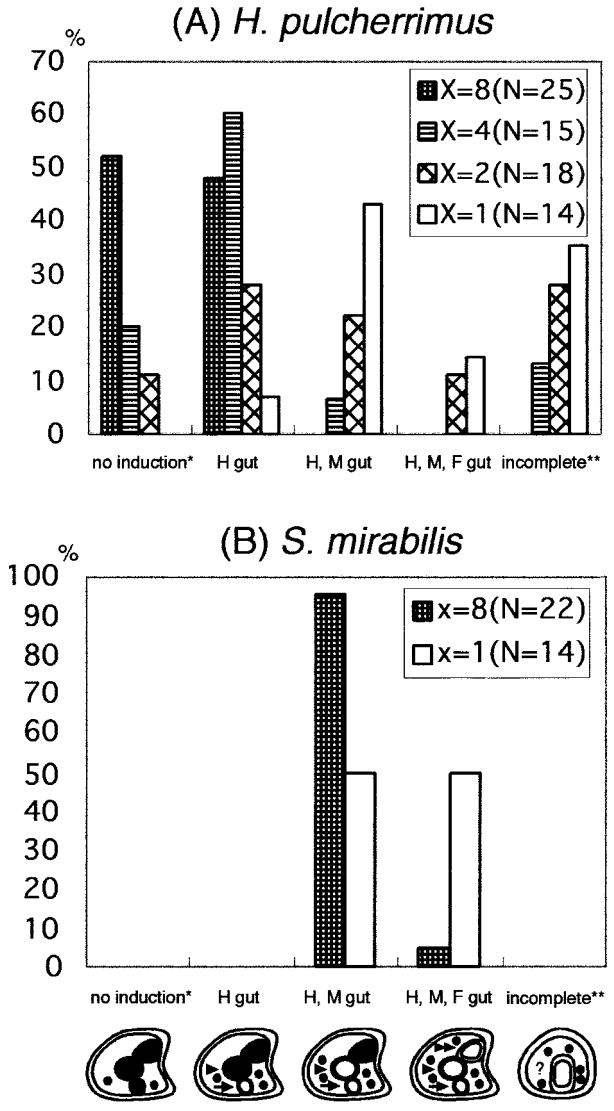

Fig. 4. Difference in inductive effects related to variations in number of *veg2* layer blastomeres of [A+V2(x)] embryos in *H. pulcherrimus* (A) and *S. mirabilis* (B). The induced region derived from animal-half cells within the digestive tract is shown diagrammatically under each group of columns. The parts of labeled *veg2* cells are painted over. Arrows, arrowheads, and double arrowheads point to the hindgut, midgut, and foregut, respectively, of the induced animal-half cells.

* The labeled *veg2* cells prevailed through all parts of the tripartite gut, so that no induction of *veg2* layer blastomeres appeared to have occurred.

** Archenteron failed to segment into a tripartite tract, resulting in the formation of an incomplete gut.

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