Developmental Biology 329 (2009) 152–166

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/developmentalbiology

Evolution of Developmental Control Mechanisms

A novel role for *dpp* in the shaping of bivalve shells revealed in a conserved molluscan developmental program

Koryu Kin, Shota Kakoi, Hiroshi Wada *

Graduate School of Life and Environmental Sciences, University of Tsukuba, 1-1-1 Ten-noudai, Tsukuba, Ibaraki 305-8572, Japan

A R T I C L E I N F O

Article history:
Received for publication 31 August 2008
Revised 3 December 2008
Accepted 12 January 2009
Available online 27 January 2009

Keywords:
Bivalve
Mollusc
Shell
Saccostrea kegaki
Cell cleavage pattern
Dpp
Engrailed
Brachyury

A B S T R A C T

During the molluscan evolution leading to the bivalves, the single dorsal shell was doubled. To elucidate the molecular developmental basis underlying this prominent morphological transition, we described the cell cleavage and expression patterns of three genes, *brachyury*, *engrailed*, and *dpp* in the Japanese spiny oyster *Saccostrea kegaki*, and examined the function of *dpp* in this species. The cleavage pattern of the *S. kegaki* embryo was nearly the same as the previously described pattern of other bivalve species, suggesting that the pattern itself is highly important for the establishment or the maintenance of the bivalve body plan. The expression pattern of a *brachyury* homolog in *S. kegaki* (*SkBra*) was similar to the pattern in gastopods even at the single cell level despite the deep divergence of gastropods and bivalves. *Engrailed* and *dpp* were previously found to be expressed around the shell anlagen in gastropods. Like that of gastropods, an *engrailed* homolog in *S. kegaki* (*SkEn*) was found to be expressed around the shell anlagen. However, the *dpp* homolog in *S. kegaki* (*SkDpp*) was expressed only in the cells along the dorsal midline. ZfBMP4 treatment experiments revealed the importance of *dpp* in establishing the characteristic shape of the bivalve shell anlagen.

Introduction

Although molluscan phylogeny is a matter of continued debate (Giribet et al., 2006; Lindberg et al., 2004), bivalves are generally thought to have evolved from a monoplacophoran-like ancestor having a single dorsal shell (Waller, 1998). The most prominent morphological change occurred during bivalve evolution is arguably the change in the number of shells, from univalvular to bivalvular. The change in shape must have been accompanied by changes in developmental processes and the underlying system that creates them. What kind of developmental changes led to the emergence of the bivalve shell is thus of considerable interest from an evolutionary, as well as a developmental perspective. Considering the widespread tinkering nature of developmental evolution (Carroll et al., 2005; Jacob, 1977; Wilkins, 2002), it is natural to assume that most of the developmental system for building a bivalve shell was employed conservatively from the developmental system for building a single dorsal shell. Some innovative changes were probably added later and, together with the formerly single-shell-forming system, constitute the bivalve-shell-forming system. Therefore, the first step toward elucidating this developmental evolution would be to determine which parts of the bivalve developmental system were present in the ancestral system and which are truly innovative components. This requires comparing bivalves to organisms that share the ancestral, single-shell-forming system, such as gastropods. In comparison to gastropods, bivalve embryogenesis has two notable features that seem to be intimately related to the formation of the bivalve shell. One is a feature in the cleavage pattern, and the other is a feature in the formation of the shell anlagen.

Both bivalves and gastropods develop by means of a “spiral cleavage” pattern (for details on spiral cleavage-based development, see, Gilbert and Raunio, 1997; Henry and Martindale, 1999). In these embryos, the first two divisions generate four blastomeres, designated A, B, C, and D, which usually correspond to the left, ventral, right, and dorsal side of the future larval body (Figs. 1A, B). These blastomeres divide unequally to generate a quartet of micromeres on the animal tier, which are designated by lowercase letters (e.g., 2d, $1a^2$; Fig. 1C). A micromere is not situated directly above its sister macromere because the orientation of the spindle is oblique with respect to the animal-vegetal axis. After the generation of the first quartet of micromeres, the macromeres continue to divide unequally to generate successive generations of animal micromere quartets. Thus, the largest cell in a spiral-cleaving embryo is usually one of the vegetal-most macromeres (Fig. 1D).

In bivalve embryos, after the second round of micromere generation, the largest cell is not one of the macromeres, but the 2d cell, which is one of the daughter cells of the 1D macromere (Fig. 1E). The 2d cell subsequently divides unequally four times, each time alternating the orientation and the relative position of the mitotic spindle in the cell. This pattern has been reported in detail for at least two bivalve species (Lilie, 1895; Meisenheimer, 1901), but not in

---

* Corresponding author. Fax: +81 29 853 4671.
E-mail address: hwada@biol.tsukuba.ac.jp (H. Wada).

0012-1606/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2009.01.021

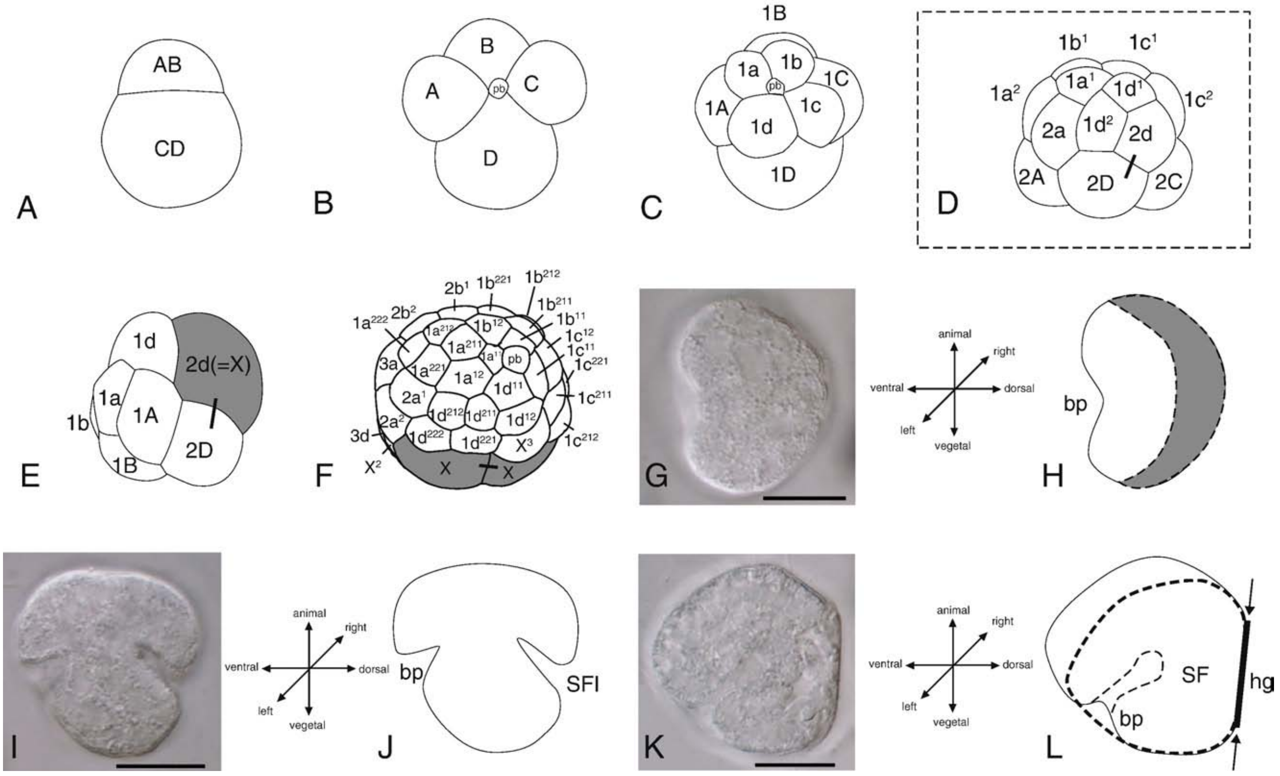

Fig. 1. Brief overview of the oyster development. (A) Animal view of the 2-cell stage *S. kegaki* embryo. (B) Animal view of the 4-cell stage. (C) Animal view of the 8-cell stage. (D) Dorsal view of the 16-cell stage *Trochus* embryo (redrawn after Dan et al., 1983) to illustrate the typical cleavage pattern of the gastropod embryo. Note that the 2d blastomere is smaller than the sister vegetal macromere, 2D. (E and F) Characteristic cleavage pattern of the bivalve embryo. The blastomere X (the largest descendant of the 2d blastomere in bivalve embryos) is colored in gray. The blastomere X is the largest blastomere in bivalve embryos. (E) Lateral view of the 9-cell stage *S. kegaki* embryo. Note that the 2d micromere is the largest cell in the embryo. (F) Animal view of the 48-cell stage *S. kegaki* embryo. The blastomere X is the first cell to divide bilaterally in the bivalve embryo. (G–L) Morphogenesis of the bivalve embryo featuring the formation of the shell field. Orientations of the images are indicated by the *xyz* axes at the right side of the images. (H, J, L) Line drawings of the (G, I, K), respectively. (G, H) Lateral view of the 7 hpf *S. kegaki* embryo. In this stage, gastrulation is going on, and the descendants of the blastomere X cover the dorsal surface of the embryo [indicated in gray, drawn after *Unio* gastrula (Lilie, 1895)]. (I, J) Lateral view of the 10 hpf *S. kegaki* embryo. In this stage both the SFI and the blastopore are clearly visible. (K, L) Lateral view of the 14 hpf *S. kegaki* embryo. In this stage the SFI has already evaginated and the shell field covers the lateral surface of the embryo. The shape of the shell field is indicated by a bold broken-line. The archenteron is represented by a thin broken-line. The hinge region is represented by a bold line and two arrows by the edge of it. bp: blastopore; hg: hinge; SFI: shell field invagination; SF: shell field. Scale bar = 20 μm.

gastropods or other molluscan species. Interestingly, the 2d cell was claimed to be the founder cell of bivalve shells (Lilie, 1895; Meisenheimer, 1901). Also, the first cell that divides bilaterally is the largest descendant of the 2d cell (Fig. 1F), not the 4d cell as in many gastropods. Given the importance of the 2d cell and its descendants in the bivalve embryo, a special notation was devised, in which the 2d cell and the largest descendant of the 2d cell are denoted as X until the bilateral cleavage stage. The first micromere generated from the X blastomere is X^1, the second is X^2, and so on.

Unfortunately, there have been virtually no detailed descriptions of cell cleavage patterns or cell lineage studies in bivalve embryos since the above-mentioned studies were conducted over a hundred years ago. Thus, we first examined the cell cleavage pattern of the embryos of the oyster *Saccostrea kegaki*, using fluorescent staining of cell boundaries and observation with a confocal laser scanning microscope (CLSM).

An understanding of cleavage patterns enabled us to determine the identity of blastomeres and to compare the lineage of specific gene expression cells between bivalve embryos and gastropod embryos. To examine the extent to which the global pattern of bivalve embryogenesis is comparable to that of gastropods, we examined the expression pattern of the gene *brachyury*. The reasons for choosing *brachyury* were two-fold: first, the *brachyury* expression pattern was examined in detail in the gastropod *Patella vulgata* (Lartillot et al., 2002), to the extent that the identities of the cells expressing *brachyury* in the early embryos were determined; second, the *brachyury* expression pattern in blastomere formation seems to have been conserved among many animal groups (Arendt et al., 2001; Technau, 2001). Comparing cell identities is important, because the differences in the cleavage patterns of bivalves and gastropods might also be related to large differences in overall developmental patterning.

We also focused on the differences in the process of shell anlagen formation. The molluscan shell anlagen are usually called the “shell field” (Kniprath, 1981), a term we use here. We will briefly describe below the process of shell field formation in bivalves, which can roughly be divided into five phases: 1) establishment of the shell-founder cell, X, 2) expansion of X descendant cells on the dorsal surface, 3) invagination of the shell field, 4) evagination of the shell field and its successive expansion to cover a whole embryo, and 5) secretion of shell matrices and calcification in the shell field.

As we noted above, the shell field is derived mainly from the founder blastomere X, which continues to divide rapidly (phase 1). After the bilateral division of X, gastrulation begins at the vegetal pole of the embryo; the archenteron is derived from the vegetal macromeres. At the time when gastrulation begins, the descendants of X cover the dorsal surface of the embryo (phase 2, Figs. 1G, H). Then these X descendant cells invaginate, and the dorsal invagination called “shell filed invagination” (SFI) (Eyster and Morse, 1984), which represents the prospective shell field, appear (phase 3, Figs. 1I, J). The SFI of bivalves is not double but single, as in gastropods. After invagination, the SFI evaginates, and the shell field, which can be morphologically distinguished from surrounding cells in terms of cell