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Gene expression patterns and pearl formation in the Japanese pearl oyster (*Pinctada fucata*): A comparison of gene expression patterns between the pearl sac and mantle tissues

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ABSTRACT

Shell matrix proteins play an important role in the regulation of shell and pearl formation in the Japanese pearl oyster (*Pinctada fucata*). There is a paucity of information on the function of these proteins and their gene expression patterns. The purpose of this study was to compare the gene expression patterns of six shell matrix proteins (*nacrein*, *msi60*, *msi31*, *n16*, *prismalin-14* and *aspein*) in the mantle center (MC) (which forms the nacreous layer) and mantle edge (ME) (which forms the prismatic layer), with those in the pearl sac (PS) (which forms the nacreous layer on the pearl surface). There was a significant correlation between the PS and MC ($P<0.05$) for the six gene expression patterns. In addition, cluster analysis showed that the expression patterns of the six genes were grouped into three clusters (nacreous cluster: *msi60* and *n16*, prismatic cluster: *msi31*, *prismalin-14* and *aspein*, and *nacrein*). These results indicate that formation of the nacreous and prismatic layers of the shell and pearl is affected by the gene expression patterns of the shell matrix proteins, and that the two genes of the nacreous cluster (*msi60* and *n16*) and the three genes of the prismatic cluster (*msi31*, *prismalin-14* and *aspein*) are regulated by unknown factors.

1. Introduction

World pearl production is about US$640 million, approximately a quarter of which is from the cultured Japanese pearl oyster (*Pinctada fucata*) (Southgate and Lucas, 2008). Pearl culture is an important industry, not only in Japan, but also in many other parts of the world.

Pearl culture comprises four steps (Wada, 1999; Southgate and Lucas, 2008): 1) pre-operative conditioning, 2) nucleus implantation, 3) post-operative care, and 4) culturing and harvest. Nucleus implantation (at the beginning of the culturing process) is the most important step; the pearl oyster is forced open, and a nucleus is implanted into the gonad with a mantle graft.

Around the nucleus, a pearl sac (PS) is formed by proliferation of the outer mantle epithelial cells of the mantle graft (e.g. Kawakami, 1953; Arnaud-Haond et al., 2007; Fang et al., 2008). In early summer, the PS in *P. fucata* is usually formed within one to two weeks after nucleus implantation (Kawakami, 1953; Machii and Nakahara, 1967). One month after implantation, the PS epithelia begin to secrete the shell matrix proteins, and then pearl formation begins (Kawakami, 1953; Machii and Nakahara, 1967). Pearl formation within the PS is affected by the shell matrix proteins secreted from the PS epithelia (Wada, 1999). However, details on the mechanisms of pearl formation and the role of the shell matrix proteins are not known.

The *P. fucata* shell consists of two mineralized layers: a nacreous layer made from aragonite, and a prismatic layer made from calcite (Sudo et al., 1997; Wada, 1999). The nacreous and prismatic layers are formed in the mantle center (MC) and mantle edge (ME), respectively, by epithelial cell secretion (Sudo et al., 1997; Wada, 1999).

A number of shell matrix proteins have been identified for shell formation in *P. fucata*: Nacrein (Miyamoto et al., 1996), MSI60 and MSI31 (Sudo et al., 1997), N16 (Samata et al., 1999), MSI7 (Zhang et al., 2003), Prismalin-14 (Suzuki et al., 2004), and Aspein (Tsukamoto et al., 2004). Nacrein has carbonic anhydrase activity and supplies the shell with carbonate ions (Miyamoto et al., 1996). MSI60, MSI31 and Prismalin-14 are insoluble proteins; MSI60 may constitute the framework of the nacreous layer, and MSI31 and Prismalin-14 that of the prismatic layer (Sudo et al., 1997; Suzuki et al., 2004). N16 induces aragonite crystals when the protein is fixed onto a substrate (Samata et al., 1999). Aspein is responsible for directed formation of calcite in the prismatic layer of the shell (Tsukamoto et al., 2004).

By analyzing the gene expression patterns of the shell matrix proteins in the mantle tissues, Takeuchi and Endo (2006) concluded that MSI60 and N16 are related to nacreous layer formation, in which the mRNAs of *msi60* and *n16* are strongly expressed in the MC. MSI31, Aspein and Prismalin-14 are related to prismatic layer formation, in which the mRNAs of *msi31*, *aspein* and *prismalin-14* are strongly expressed in the ME.

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Takeuchi and Endo (2006) did not compare the gene expression patterns in the PS with those in the mantle tissues. Although Inoue et al. (2009) and Wang et al. (2009) reported the gene expression patterns of the shell matrix proteins in the PS, they did not consider the mantle tissues. Wang et al. (2009) did consider the mantle tissues, although they did not observe the quality of the pearl that was formed in the PS.

When considering the quality of the pearl that is formed in the PS, it is necessary to compare the gene expression patterns of shell matrix proteins in the MC and ME with those in the PS. If the gene expression patterns affect formation of the two mineralized layers, the gene expression patterns of the PS that result in formation of high quality pearls with a surface layer composed of only a nacreous layer should be consistent with those in the MC. The purpose of our study was to compare the gene expression level patterns of shell matrix proteins in the MC and ME with those in the PS to identify the mechanisms responsible for formation of the two mineralized layers in the shell and pearl.

2. Materials and methods

2.1. Donor and host pearl oysters

Host pearl oysters (about 3 years of age) were "pre-operative conditioned" for two weeks prior to nucleus implantation, and mantle grafts (2–3 mm^2) were cut from the mantle pallial of donor pearl oysters (50–60 mm hinge length, 2.5 years of age). A total of 233 individuals were implanted with one nucleus each (about 7 mm diameter) on either 7 or 29 July 2008. Implantations were carried out by an experienced (i.e. more than 20 years) technician. After two weeks' post-operative care, the host pearl oysters were cultured for about two months on rafts in offshore waters in Ago Bay, Japan. The mantle graft and culturing methods for both the donor and host oysters were based on those in Acosta-Salmon and Southgate (2006) and Southgate and Lucas (2008).

2.2. Pearl quality and tissue sampling

A total of 131 oysters that had been implanted with nuclei were harvested on either 1 September or 14 October 2008. The soft tissues including the pearls of 131 individuals were fixed for about 24 h in 99% ethanol and then transferred to new 99% ethanol at 4 °C.

The fixed soft tissues were dissected and the harvested pearls classified into four grades (Grade I, high quality; to Grade IV, low quality) using the method described by Munetomo (1994). Of the 131 individuals that were harvested, only 37 produced high quality, Grade I pearls. Of those 37 individuals, four (60–67 mm in hinge length) that produced typical high quality pearls (Fig. 1a) were selected for tissue

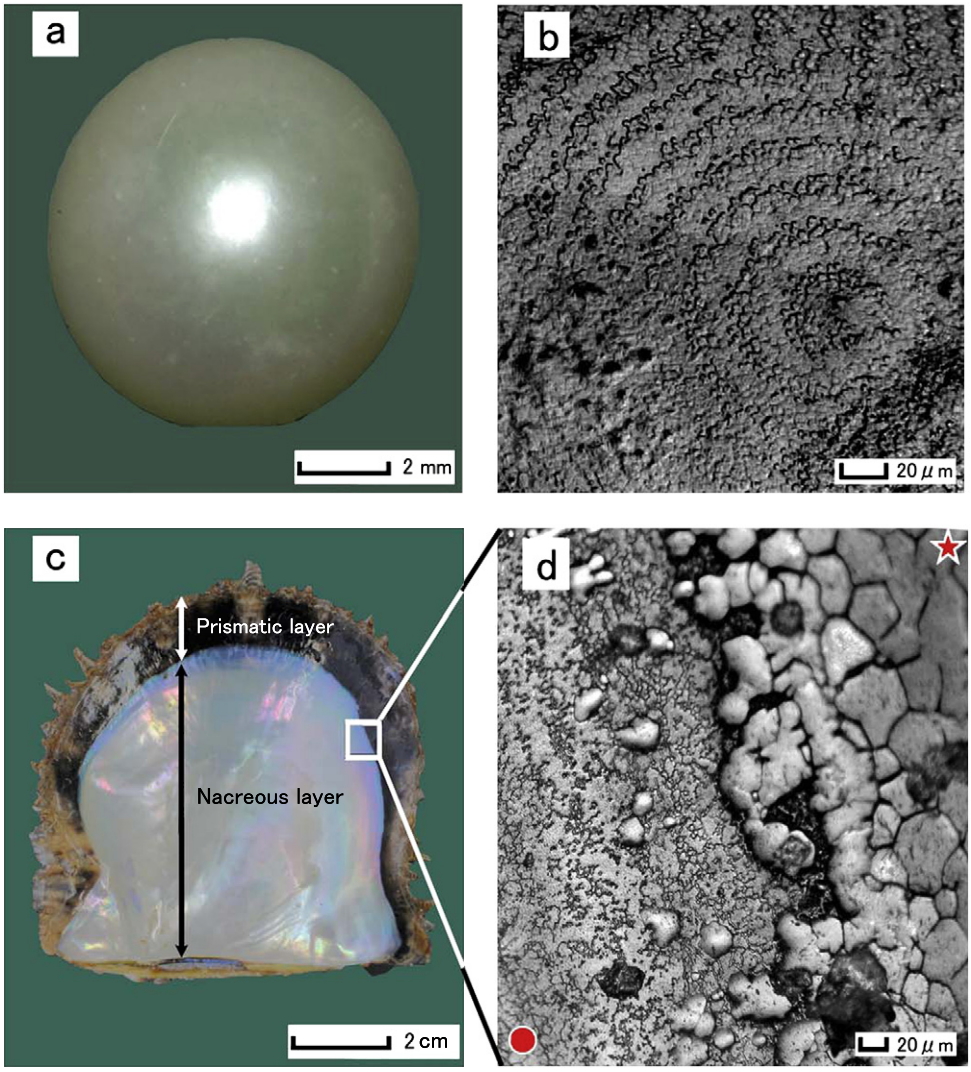

Fig. 1. Photographic and digital microscopic images (KEYENCE, VHX-1000) of a typical high quality pearl and the shell surface; a, typical high quality pearl harvested in this study; b, close-up image of a typical high quality pearl surface (500×), only nacreous layer formation is observed; c, inner shell surface; d, close-up image of the inner shell surface (500×), nucleus and prismatic layer formations are indicated by the red circle and star, respectively.