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Organic matrices regulating the biomineralization -Structural and functional analyses of Pif in the nacreous layer-

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A wide variety of organisms use biominerals to generate hard tissues that function in the maintenance of body structure, protection from enemies, sensing magnetic fields and gravity, and the storage of minerals. In addition to their mineral components, biominerals contain a small amount of organic matrices that likely play important roles in biomineral formation. These organic matrices have been identified from a wide range of biominerals, such as bones, teeth, coccoliths, otoliths, the exoskeleton of crustaceans, seashells, eggshells, the exoskeleton of corals and bacterial magnetite. Acidic matrices, which are necessary for mineralization, are included in these biominerals. These acidic matrices can interact specifically with the mineral's crystal surface to induce axis-oriented nucleation, intercalate into the crystal lattice, determine the mineral phase, or function as the framework of biomineral formation.

In molluscan shells, these organic matrices play a role in the formation of calcium carbonate crystals. In turn, these crystals are necessary for the formation of the nacreous layer contained in the pearl oyster shell, which is used for production of jewelry pearls. Within the nacreous layer, aragonite calcium carbonate crystals are sandwiched between sheets of the organic matrix, and the c-axis of the crystals is perpendicular to the shell surface. Within molluscan shells, the major components of organic matrices are Asp-rich calcium-binding proteins and chitin. Previous studies suggested that unidentified acidic proteins induce aragonite formation and control the direction of the c-axis.

In 1960, Watabe and Wilbur first reported that the whole organic matrices extracted from the nacreous layer induced aragonite crystal formation. Later, it was suggested that Asp amino acid residues of the organic matrices interact with calcium atoms in the calcium carbonate to regulate its polymorph. Recent studies imply that a few organic matrices in the nacreous layer play important roles in the formation of such characteristic aragonite crystals. Although a number of matrix proteins have been identified from various mollusk shells, any fully identified proteins which induce aragonite crystal formation characteristic of nacre as mentioned above have not yet been found out, nor has any Asp-rich acidic macromolecule been discovered from the nacreous layer. Therefore, the molecular mechanism of formation of the characteristic structure of the nacreous layer remains unknown. To clarify the formation mechanism of the nacreous layer, we searched for a key molecule in the nacreous layer and identified a novel acidic matrix protein, Pif, in *P. fucata* that specifically binds to aragonite crystals. The Pif cDNA encoded a precursor protein, which gave Pif 97 and Pif 80 posttranslationally. The results of immunolocalization, a knockdown experiment by RNAi, *in vitro* calcium carbonate crystallization studies and the existences of orthologs in closely related species strongly indicated that Pif regulates the nacre formation such as stacked compartment structure, polymorph switching and crystal alignment.

Keywords: aragonite, nacreous layer, Pinctada fucata, matrix protein