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Author(s)	DHAR, Dipanjana
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Abstract of Doctoral Dissertation

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Title of Doctoral Dissertation

Elucidation of mitogenomic adaptation and structural characteristics of big defensin in molluscs using bioinformatics and computational biology
(バイオインフォマティクスと計算生物学を用いた軟体動物におけるミトゲノムの適応とビッグディフェンシンの構造特性の解明)

The phylum Mollusca constitutes an ubiquitous, heterogeneous, ecologically and economically important group of invertebrates that function as ecosystem engineers. Although the most conspicuous symptom of any ecosystem deterioration is the decline or disappearance of sensitive species, some organisms such as molluscs display an unusual resilience towards environmental changes. Marine molluscs, that survive in the challenging environments of different oceanic zones, are ideal systems for studying stress adaptation. The understanding of adaptive evolution of mitochondrial genomes in molluscs and structural characterization of mollusc defensin protein which forms the essential component of the innate immunity, have formed the major baseline for my PhD studies in Hokkaido University.

Mitochondria are known to be critical for energy homeostasis and changes in environmental factors result in their dysfunction and consequent injury to the organism. Mitochondrial proteins and mitochondria-derived stress signals regulate both oxidative phosphorylation and innate immune response. Maintenance of mitochondrial integrity and signaling are important for cellular homeostasis and survival. Evolutionary changes in the constituent residues of the mitochondrial proteins might have an impact on their functional domains, such as the regions lining the proton translocation channel or subunit interacting sites, thereby allowing animals to adapt to challenging environments. Therefore, the aim of this study is to estimate selection pressure acting on mitochondrial proteins that could provide insights into the adaptive evolution of the mitochondrial genome.

Chapter 1 provides insight into the molecular mechanisms underlying the adaptive strategies of polyplacophorans to the intertidal habitat from a mitochondrial perspective. The intertidal zone is one of the most stressful environments, with extreme shifts in temperature, salinity, pH and oxygen concentration. Chitons (Polyplacophora) are marine molluscs with a fossil record extending back to the Early Cambrian. They predominantly inhabit the intertidal zone and are able to maintain mitochondrial homeostasis regardless of regular oscillations of immersion and emersion, as well as extreme alternations of temperature, salinity, pH and hydrodynamic forces in their environment. Here, I used mitochondrial genetic components from seven chitons of the intertidal zone to infer phylogenetic relationships. Selection analyses on individual protein-coding genes (PCGs) were performed to identify and map potentially adaptive residues in the modelled structures of the mitochondrial respiratory chain complexes. The results showed significant amino acid changes in sites under diversifying selection for all the PCGs, indicating that the mitochondrial genome in chitons is undergoing adaptive evolution. Such sites were observed in the proton pump as well as in the

translocation channel of the transmembrane helices and the surrounding loop regions, thus implying functional modification of the mitochondrial proteins essential for survival in the dynamic environment of the intertidal zone.

Chapter 2 sheds light into the mitogenomic adaptations of intertidal and deep sea gastropods. Of all the classes of the phylum Mollusca, gastropods have radiated into marine, freshwater and terrestrial habitats, thus successfully adapting themselves to thrive in changing environmental conditions. In order to withstand the constant fluctuations in temperature, salinity and shifts in oxygen concentration of the intertidal zone, the gastropods inhabiting here rely on a modified and adaptive energy metabolism. The same is applicable for gastropods living in the deep sea environment, which is characterized by high hydrostatic pressure, low oxygen concentrations and abundance of heavy metals. Therefore, survival of these organisms may be correlated to their adaptive mitochondrial genome which serves as the principal site for energy metabolism and production in the cell. Here, I estimated selection pressure acting on the mitochondrial PCGs of 13 intertidal and 2 deep sea gastropods based on site and branch-site specific models. The results exhibited higher number of sites under diversifying selection for the mitochondrial PCGs of intertidal gastropods compared to deep sea species. Overall, this study focusses on the adaptive mitogenome evolution of marine gastropods for survival in the dynamic environments of the intertidal zone as well as deep sea.

Chapter 3 deals with the structural characterization of big defensin of *Crassostrea gigas* through various bioinformatic tools and molecular dynamics simulation. Defensins are antimicrobial peptides consisting of three or four intramolecular disulphide bonds which are formed by six or eight cysteine residues, respectively, in a complex array of two or three antiparallel β-sheets with or without an α-helix structure. They are produced by a vast range of organisms and are constitutively expressed or induced in various tissues in response to different stimuli like infection, injury and other inflammatory factors. Two classes of invertebrate defensin exist, namely CSαβ defensin and big defensin, the latter being predominantly present in molluscs. Interestingly, an invertebrate big defensin gene has been hypothesized as the most probable ancestor of vertebrate β-defensins. In this study, conserved residues were identified for both the big defensin and β-defensin. I performed *in silico* mutation on conserved amino acid positions of the β-defensin-like domain to understand the effect of mutations on the structure and function of big defensin. Molecular dynamics simulations were performed on wild-type and two mutants (R64A and E71A) for 100 ns to assess the structural stability and conformational dynamics of the protein in its wild-type and mutated form. The aforementioned mutations have been identified as deleterious as well as destabilizing for the three-dimensional structure of big defensin, as revealed by bioinformatic tool analyses. Changes in amino acid network with interacting residues and aggregation propensity further support the structural basis of big defensin upon mutation. 100 ns molecular dynamics simulations of wild-type, R64A and E71A structures revealed significant conformational changes in the case of mutants. Therefore, this study aims to unveil the detailed structural characteristics of a molluscan defensin through a computational approach. It will further enhance the current knowledge of this protein for application in therapeutics and other aspects of protein engineering.