HOKKAIDO UNIVERSITY

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Doctoral Dissertation Evaluation Review

Degree requested: Doctor of Science Applicant's name: Dipanjana Dhar

Examiner :

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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)**

Results of Evaluation of the Doctoral Dissertation (Report)

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 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.

The author performed research using several species of mollusks from deep see and intertidal habitats using bioinformatics methods aiming at to estimate selection pressure acting on mitochondrial proteins that could provide insights into the adaptive evolution of the mitochondrial genome. The results showed that intertidal chitons have significant amino acid changes in sites under diversifying selection for all the protein – coding genes (PCGs). This was observed in the proton pump as well as in the translocation channel of the transmembrane helices and the surrounding loop regions. These data shows that chitons' mitochondrial genome is undergoing adaptive evolution essential for survival in the dynamic environment of the intertidal zone.

Further on, the study of mitochondrial PCGs from deep-sea and intertidal gastropods showed that intertidal species have a higher number of sites under diversifying selection compared to deep sea species. These results show that although marine gastropods mitogenomes is adapting for survival in extreme conditions of the deep-sea and the intertidal, the later show a higher degree of evolutionary dynamics than those from the deep-see probably because of a higher instability of the intertidal environments.

Finally, the author focused on the characterization of the big defensing of *Crassostrea gigas* (Pacific oyster) using molecular dynamics simulation. These proteins are responsible for the immunity and response of mollusks in case of injury, infections and parasite inflammation. Results showed that induced mutations in several conserved amino-acid positions of the β-defensin-like domain had deleterious and destabilizing effects in the three-dimensional structure of big defensin, leading to the loss of many functional characteristics of this protein complex.

In conclusion, the author has new findings in the field of mollusk adaptation to environmental stress using bioinformatics and protein modeling. Results provide insights into the molecular mechanisms used for stress adaptation in intertidal chitons and gastropods from a mitochondrial perspective. This study also provides the first structural characterization of the big defensin proteins in *Crassostrea gigas*, an economical important oyster. Defensin proteins are responsible for antimicrobial resistance and as such the knowledge of their structure and possible effects of mutations are very important both scientifically and for the practice of aquaculture.

Overall, the work findings will contribute to a better knowledge of the adaptation mechanisms in intertidal mollusks especially when anthropogenic impacts in coastal areas are increasing. It also offers new insights concerning the detailed structural characteristics of molluscan defensin proteins through the use of bioinformatics tools. Finally, findings can be further in protein engineering and its application in therapeutics and aquaculture.

Therefore, we acknowledge that the author is qualified to be granted a Doctorate of Science from Hokkaido University.