Environmental adaptation mechanism of marine annelids

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General Introduction

Marine annelids are segmented worms found in all marine environments and play an important role in maintaining of marine ecosystems. Therefore, marine annelids are considered to have astonishing abilities for environmental adaptation. The various cases of environmental adaptation of marine annelids have so far been reported. However, there is little explanation of their environmental adaptation mechanism from the molecular biological aspects. In this study, I intend to elucidate the environmental adaptation mechanism of marine annelid focusing on that of two species, deep-sea hydrothermal vent-endemic worm, *Paralvinella hessleri* and organically polluted area-endemic worm, *Capitella teleta*. Here, I showed that *P. hessleri* has high chemical sensitivity mediated by transient receptor potential (TRP) channels to minimize the exposure of toxic hydrothermal fluid, and *C. teleta* escape from their burrow in hypoxia *via* detection of it by TRPA homologue with improving ability to acquire oxygen by changing molecular species of globin genes.

Chapter 1
High chemical sensitivity of deep-sea hydrothermal vent-endemic annelid, *Paralvinella hessleri.*

The environments around deep-sea hydrothermal vents are very harsh conditions for organisms due to the possibility of exposure to highly toxic compounds and extremely hot venting there. Alvinellid worms (Annelida) are among the organisms best adapted to high-temperature and oxidatively stressful venting regions. Although intensive studies of the adaptation of these worms to the environments of hydrothermal vents have been made, little is known about the worms' sensory adaptation to the severe chemical conditions there. To examine the sensitivity of the vent-endemic worm *P. hessleri* to low pH and oxidative stress, I determined the concentration of acetic acid and hydrogen peroxide that induced avoidance behavior of this worm, and compared these concentrations to those obtained for related species inhabiting intertidal zones, *Thelepus* sp. The concentrations of the chemicals that induced avoidance behavior of *P. hessleri* were 10-100 times lower than those for *Thelepus* sp. To identify the receptors for these chemicals, chemical avoidance tests were performed with the addition of ruthenium red, a blocker of transient receptor potential (TRP) channels. This treatment suppressed the chemical avoidance behavior of *P. hessleri*, which suggests that TRP channels are involved in the chemical avoidance behavior of this species. These results revealed for the first time hypersensitive detection systems for acids and for oxidative stress in the vent-endemic worm *P. hessleri*, possibly mediated by TRP channels, suggesting that such sensory systems may have facilitated the adaptation of this organism to harsh vent environments.

Chapter 2
Exploration of the hypoxia sensor for behavioral response in marine annelid, *Capitella teleta.*

Hypoxia often occurs in summer and causes deleterious effects on marine benthic animals. A marine annelid, *C. teleta*, may be able to overcome hypoxia, as shown by the fact that it inhabits sediments rich in organic matter, where severe hypoxia is often observed. To understand how this species adapts to the environment, I focused on its hypoxia sensor, and showed that TRPA homologue is a possible contributor to hypoxia detection in *C. teleta*. To examine the involvement of TRPA1, known as hypoxia sensor for mammals, in the response of *C. teleta* to hypoxia, *C. teleta* was exposed to hypoxic water with or without a TRPA1-specific inhibitor, A-967079. Hypoxic stimulation induced escape behavior of *C. teleta* from the sediment, and this behavior was suppressed by the inhibitor. The cloned TRPA gene from *C. teleta* was phylogenetically categorized into *TRPAbasal*, and its deduced amino acid sequence contains an oxygen-dependent degradation domain, which is important for the detection of hypoxia. Whole-mount *in situ* hybridization analysis showed that the gene was transcribed in the prostomium, where sensing functions are localized. These results suggest that the worm has a hypoxia-sensing system possibly utilizing CtTRPAbasal, and this system contributes to expanding the organism's niches in environments with low dissolved oxygen concentration by detecting whether hypoxia exceeds a level that would imperil its survival.

Chapter 3
Transcriptomic analyses reveal hypoxia-inducing responses in marine annelid, *Capitella teleta.*

In previous chapter, I demonstrated that the hypoxia-escaping behavior of *C. teleta*, however the mechanism to survive in hypoxic condition remains unclear. In this chapter, to reveal the molecular basis supporting their adaptability to hypoxic condition, RNA-seq analysis were conducted. Here, I showed that *C. teleta* scavenges oxidative stress from hypoxia and reoxygenation, and changes the set of globin genes in response to hypoxia. I also showed the possibility that hypoxia-inducing responses differ from the growth stages. The number of differentially expressed gene was larger in large worm and that common in both growth stages was smaller than that specific to each growth stages. This suggests that the larger would be more influenced by hypoxia and the responses to hypoxia differ from the growth stages. The genes showing high differential expression level by hypoxia were heat shock protein and globin. Heat shock protein seems to play a role for preventing functional protein from oxidative stress produced by hypoxia and reoxygenetion. Globin genes showed complex expression pattern, i.e. showed not only high upregulation, but also high downregulation. To examine the function of globin genes in hypoxia in detail, phylogenic analysis and clustering analysis were conducted. These analyses demonstrated that deduced red blood cell hemoglobin shows highly differential expression level in response to hypoxia and the set of expression in molecular species of globin gene were different from the growth stages and environmental DO level. This results suggest that *C. teleta* would switch the expression pattern of globin gene responding hypoxia to improves oxygen availability.

General discussion

I demonstrated that *P. hessleri* escapes acids and oxidative stress with higher sensitivity than shallow species does, and that *C. teleta* evacuates from the burrow in response to hypoxia. Pharmacological tests showed that these avoidance behaviors from environmental stresses are mediated by TRP channels. In addition to this, I illustrated the comprehensive transcriptomic responses to hypoxia in *C. teleta*, i.e. they improve the ability to prevent functional proteins from oxidative stresses produced by hypoxia and reoxygenation, and that to acquire oxygen by changing molecular species of globins. Environmental adaptations of organisms have often been considered from the three point of view, morphological, physiological and behavioral adaptation. However, this study indicates that sensory adaptation also plays an important role in environmental adaption because the function for sensing environmental factors is to perceive precisely the range of survival limits and to regulate physiology and behavior in response to the environmental changes.

In decades, marine environments have been globally changed by anthropogenic activity. Ocean acidification, eutrophication, warming and hypoxia are observed and may cause various effects on marine ecosystems. The achievement of this study demonstrating molecular responses of the major maintainer in seafloor, marine annelid, to environmental factors may help to forecast the impacts of the change in marine environments on marine ecosystems.