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MINIREVIEW

Transcriptomic response to stress in marine bivalves

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Abstract

Marine bivalves have a set of unique capabilities to adapt to the complicated conditions owing to their habitats, living habits and feeding ways. Meanwhile, marine bivalves can be the biosensors to monitor the quality of the intertidal zones or other habitats. It is interesting for every biologist to find out the mechanisms by which organisms adapt to environmental challenges and the factors limiting their adaptive capacities. The development of biotechnology over the past few decades has provided biologists with a vast repertoire of biosensors that allow testing mRNA expression in response to environmental factors. This minireview is focused on the transcriptomic responses to abiotic and biotic stressors in bivalves and the relative methods to provide new perspectives as well as improve applications for bivalve biomonitoring studies.

Key Words: transcriptome; stress; bivalve

Introduction

Marine bivalves are an important component of the ecosystem and biodiversity (Dame, 2011), which have abundant species distributed worldwide from the intertidal zones to hydrothermal vents and cold seeps (Bettencourt *et al.*, 2010; Boutet *et al.*, 2011; Egas *et al.*, 2012). Bivalve cultivation is one of the most important aquaculture industries globally (Forrest *et al.*, 2009; Pawiro 2010). Furthermore, marine bivalves possess the unique adaptation to problematic surroundings. In light of the important status of marine bivalves in ecosystem and economy and their high adaptations, they are the valuable organisms to be investigated about their molecular mechanism responding to the variable environment. It is also meaningful to find relative gene expression index in marine bivalves to be the monitoring standard of the surroundings. Owing to no model organism in marine bivalves and the repetitive organization of the non-coding fraction in their genome, as well as their size, the development of the genome and transcriptome of them make a slow progress. Thanks to the technical advance, marine bivalves have acquired growing concerns and their genomic databases have enriched increasingly, such as MytiBase (http://mussel.cribi.unipd.it) for *Mytilus galloprovincialis* and DeepSeaVent (http://transcriptomics.biocant.pt:8080/deepSeaVent) for *Bathymodiolus azoricus*.

Marine bivalves commonly inhabit variable and unstable conditions and most are the crisscross between anthropic zone and the nature. Complex ecological habitats bring about all kinds of stresses in the lives of bivalves and determine their distributions and abundance. These stresses are mostly from some reasons as follows.

Firstly, intertidal zone that is one of the most important habitats for bivalves experiences large and sometimes rapid fluctuations caused by tidal fluctuations, rain and freshwater run-off (Shumway, 1977). Owing to this, intertidal bivalves are mostly exposed to multiple stressors including periodic hypoxia, hyposaline, temperature fluctuations and pollution (Ivanina *et al.*, 2012). Meanwhile, in the recent past, anthropogenic inputs of contaminants, and combustion of fossil fuels and deposition of metals make the condition further complicated (Doney, 2010). Furthermore, some important cultured bivalve species, such as Pacific oyster and blue mussel, are subject to the "summer mortality" (Tremblay *et al.*, 1998a; Tremblay *et al.*, 1998b; Xiao *et al.*, 2005; Samain *et al.*, 2007; Lynch *et al.*, 2012). Summer mortality has been reported to occur during the summer months in several countries (Myrand and Gaudreault, 1995; Cotter *et al.*, 2010; Fleury and Huvet, 2012). This phenomenon is multifactorial, resulting from a complex interaction between organisms, environment and pathogens (Samain *et al.*, 2008).

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Secondly, climate changes will affect temperature extremes and averages, and hyposaline conditions in coastal areas due to extreme precipitation events and oceanic pH (Tomanek, 2012). Meanwhile, the changes of the environment can affect the distribution and abundance of the native species (Johnson *et al.*, 2011), even lead to change the competitive interactions between invasive and native species (Lockwood *et al.*, 2010; Lockwood *et al.*, 2011).

Lastly, it puts down to their living habits and feeding ways of themselves. Most bivalves are filter-feeding and their mobility is not great. They cannot escape the stresses by moving away quickly and have to adjust to the changing surroundings other than swimming organisms. It is more valuable to decipher the mechanisms of their unique capacities to adjust to the variable environment.

There are kinds of reasons to induce the responses of the marine bivalves, and the factors involved in the stresses have multiple modes of interaction. Responses to the environmental stresses on the transcriptomic level are complicated and hard to explain by single gene or pathway. Actually, even against the single stress, there are a lot of genes participated and the complex networks between genes and pathways. Here we provide an overview about the transcriptomic responses to abiotic and biotic stressors in marine bivalves and the development of the relative technological methods to provide promising perspectives for a better comprehension of the mechanisms.

Abiotic stress responses

Temperature

Temperature has been shown to be one of the most important determinants of survival, growth and reproduction (Helmuth *et al.*, 2006; Menge *et al.*, 2008). Understanding the underlying mechanisms by temperature driving organismal responses and physiological performance is becoming increasingly imperative as climate change alters habitat temperature (Somero, 2010). Meanwhile, sessile inhabitants of marine intertidal environments commonly face heat stress which is an important incentive in “summer mortality” (Tremblay *et al.*, 1998a; Soletchnik *et al.*, 2005).

The largest changes in gene expression in response to heat-stress were among genes that encoded the molecular chaperone heat shock protein 70 (Hsp70) and his family (Lang *et al.*, 2009; Lockwood *et al.*, 2010; Chapman *et al.*, 2011). The increased expression of molecular chaperones is a primary component of the cellular stress response and a key indicator of environment stress (Lockwood *et al.*, 2010). Hsps, especially Hsp70 and Hsp90, can protect cells and organisms from thermal damages (Zhao and Jones, 2012) and are well known as molecular chaperones that help in the refolding of misfolded proteins and assist in their elimination if they become irreversibly damaged (Zhao and Jones, 2012). Peroxinectin is up-regulated during exposure to elevated temperature, which is reported in oysters (Lang *et al.*, 2009; Chapman *et al.*, 2011). Peroxinectin may perform adhesive and defensive functions (Lang *et al.*, 2009), owing to higher temperature negatively impacting resistance to bacteria (Chapman *et al.*, 2011). Transcriptomic responses to heat stress are, in part, characterized by the up-regulation at 32 ^{o}C of genes involved in proteolysis, which was specific to ubiquitin-mediated proteolysis in *Mytilus trossulus* (Lockwood *et al.*, 2010). Proteolysis is the directed degradation of proteins and in the context of environmental stress serves to degrade and remove permanently denatured proteins, an indicator of severe cellular stress (Dahlhoff, 2004; Kültz, 2005). Heat shock can also affect the genes related to growth and reproduction. The relative genes such as suppressor of cytokine signaling-2, collagen, were up-regulated during heat stress (Lang *et al.*, 2009).

Salinity

In intertidal zones, the salinity of seawater can strongly vary from nearly fresh water to highly saline (Meng *et al.*, 2013). Some bivalves, such as the Pacific oysters, are able to survive in a range of salinity from 10‰ to 35‰ (Pauley *et al.*, 1988). For mussels, the salinity may determine the outcome of competition between native and invasive mussels (Lockwood *et al.*, 2011).

During hypo-osmotic shock, all reported changes in gene expression mainly focus on osmoregulation and osmotic stress signaling (Lockwood *et al.*, 2011; Zhao *et al.*, 2012; Meng *et al.*, 2013). Osmoregulation influences the up-regulation of genes encoding ion channel (Lockwood *et al.*, 2011; Zhao *et al.*, 2012; Meng *et al.*, 2013) and free amino acid (FAA) metabolic key enzyme (Meng *et al.*, 2013), as well as the down-regulation of the ion and amino acid transporter genes (Lockwood *et al.*, 2011). Salt stress signal transduction pathways including calcium signaling cascade and phosphorylation regulation were observed to be up-regulated (Zhao *et al.*, 2012; Meng *et al.*, 2013). Furthermore, FAA metabolic pathways (including those for glycine, alanine, beta-alanine, arginine, proline, and taurine) were activated, altering the osmotic status in the oysters (Meng *et al.*, 2013). In the study of mussels, ornithine decarboxylase, serving as the key regulatory enzyme in polyamine synthesis was highlighted, owing to different expression between two mussels, which regulated cell growth and cell viability and whose disruption can cause cancer or apoptosis (Lockwood *et al.*, 2011). Moreover, many other types of metabolism, including immune responses and apoptosis, were shown to be enriched in the KEGG pathway analysis.

Metals and chemicals

Most species of marine bivalves have a long history as sentinel organisms for monitoring the status of marine ecosystems (Dondero *et al.*, 2006; Hines *et al.*, 2007; Milan *et al.*, 2011; Varotto *et al.*, 2013). Heavy metals including Cu^{2+}, Cd^{2+}, Hg^{2+}, Ni^{2+}, participate in the biological cycles of different groups of organisms (Zapata *et al.*, 2009), affecting their distribution and abundance (Kudo *et al.*, 1996). In previous studies, copper gives rise to the differentially expressed genes involved in the respiratory chain and stress response, development

and differentiation, cytoskeleton in *Chilean scallop* (Zapata *et al.*, 2009). Metallothionein is the most well-known antioxidant that protects against metal toxicity, and was verified in marine bivalves (Venier *et al.*, 2006; Dondero *et al.*, 2011; Varotto *et al.*, 2013). Moreover, Nickle modulated proliferation, growth and apoptosis as the same as copper, and highly regulated the genes encoding lipid metabolism (Dondero *et al.*, 2011).

Organic contaminants elicited defensin C and the defender against cell death 1 gene (whose defective function causes apoptotic death) that were under-represented in mussels other than from heavy metals (Venier *et al.*, 2006). Similarly, in mussels, Chlorpyrifos decreased in acetylcholinesterase activity in the gills markedly, and up-regulated the chitinase activity, which play a role in digestion and participate in the innate immune response (Dondero *et al.*, 2011). In addition, studies focused on the responses of *Mytilus edulis* to benzo[α]pyrene found that organic substance mostly disrupt the cellular redox status (Brown *et al.*, 2006). Notably, gene expression levels primarily depend on the functional specificity of cells composing different organs and tissues (Venier *et al.*, 2006). So that, to different tissues, the transcriptional responses to the pollution are specific.

Hypoxia

Oxygen deficiency is a common stressor in estuarine and coastal environments. Intertidal molluscs are among the animal champions of hypoxia tolerance owing to a suite of metabolic adaptions that allows them to survive prolonged periods without oxygen. They adapt to the fluctuation of oxygen by changing energy management and resource utilizations.

In *Crassostrea gigas*, which exposed to hypoxia, an overall transcriptome study indicated many genes coding for enzymes involved in antioxidant defense and reactive oxygen species detoxification for the cellular redox balance except for genes related to stress exhibited over-expressed (Sussarellu *et al.*, 2010). Meanwhile, some literatures were to characterize the some genes in bivalves and to define their potential regulation in the hypoxic response. Hypoxia-inducible factor-1 (HF-1), as the key regulator of oxygen homeostasis in aerobic organisms under hypoxia, had been verified to play a critical role in reactive oxygen species (ROS) production of hemocytes in *C. gigas* (Choi *et al.*, 2013). And likewise, AMP-activated protein kinase α (AMPKα) was showed to participate in the metabolic response during hypoxia in the smooth muscle of *C. gigas* (Guévélou et al., 2013). However, *Crassostrea virginica* has both a better tissue aerobic capacity to compensate for reduced oxygen availability and a lower sensitivity to hypoxia than *C. gigas*, with a compensatory increase in activities of citrate synthase and cytochrome c oxidase after 2 weeks of hypoxia (Ivanina *et al.*, 2011).

Other abiotic stresses

The different conditions of different vertical locations in intertidal zone depend on the tides. The tidal fluctuations changed many environmental factors such as temperature and food availability of the surroundings. Researchers caught this natural phenomenon and showed that low intertidal mussels altered their physiology very little with respect to the tide cycle, and mid-intertidal and high intertidal mussels reduced the gene expressions involved in metabolic processes (Place *et al.*, 2012). Especially, in high intertidal zones, pathways associated with protein rescue, cellular repair and protein degradation and oxidative stress were activated (Place *et al.*, 2012).

Biotic stresses

Biotic stress mainly refers to the stress that occurs as a result of damage to plants and animals by other living organisms such as bacteria, viruses, parasites and microalgae. Marine bivalves harbor an abundant and diverse microflora on their surface or inside their tissues. With evolution, marine bivalves have developed effective systems for maintaining their homeostasis and for controlling potentially harmful and pathogenic microorganisms and microalgae.

The current literature shows that bivalves eliminate or limit the development of the microorganisms through different innate immune response in combination with other cellular mechanism, such as the apoptotic pathway by transcriptomic analysis (Wang *et al.*, 2010; De Lorgeril *et al.*, 2011; Morga *et al.*, 2011; Brulle *et al.*, 2012; Moreira *et al.*, 2012a; Moreira *et al.*, 2012b). In flat oyster (*Ostrea edulis*), Fas-ligand that was involved in the immune response against the parasite *Bonamia ostreae* was observed up-regulated. Meanwhile, according to the previous results, Fas-ligand is also associated in the apoptosis pathway with the inhibitors of apoptosis proteins (Morga *et al.*, 2011). Furthermore, apoptosis as autophagy can be triggered by reactive oxygen species (ROS), and up-regulated genes are related to respiratory chain and particularly in ROS production (Wang *et al.*, 2010; De Lorgeril *et al.*, 2011). Above all, the apoptosis pathway is the most important response to the biotic stresses. In addition to the apoptosis pathway, other immune-related genes were reported, such as ferritin and lysozyme, several immune pathways and processes including the toll-like signaling pathway and the complement cascade (Wang *et al.*, 2010; De Lorgeril *et al.*, 2011; Moreira *et al.*, 2012).

Except for the immune response to the microorganisms, some bivalves exposed to *Vibrio spp*. (Gestal *et al.*, 2007; Brulle *et al.*, 2012; Moreira *et al.*, 2012b), parasite including *Bonamia* (Martín-Gómez *et al.*, 2012) and *Perkinsus marinus* (Tanguy *et al.*, 2004) were elicited that such cytotoxic response led to the rearrangement of the cytoskeleton. Cytoskeleton is important in phagocytosis, and all phagocytosis processes are driven by rearrangement of the actin cytoskeleton (May *et al.*, 2001).

Besides the microorganisms, dinoflagellates and other microalgae can produce a wide spectrum of toxic molecules. During seasonal harmful algae blooms (HABs), many filter-feeding bivalves can

accumulate phycotoxins at extremely high levels, thus representing a serious threat to human health.

Until now, a few researches about the transcriptome of marine bivalve induced by harmful algae have been reported. Manfrin *et al.*, 2010 studied the molecular mechanism that *M. galloprovincialis* exposed to okadaic acid (OA) which is a lipophilic toxin. Its results indicated that the effects of OA mostly concentrated in genes related to stress response, apoptosis and cell structure function (Manfrin *et al.*, 2010). These variations were also observed in the study that evaluated *C. gigas* hemocytes responses to purified PbTx-2 *in vitro* (Mello *et al.*, 2012). In both researches, there were no genes associated to immune or antioxidant, which needs more experiments to be tested and verified.

Interactive stresses

Previous researches tended to control a single variable to discuss the transcriptomic responses and acquire the master genes, because the single variable is easier to control under the laboratory conditions. However, the natural circumstances consist of multifactor and are multivariable other than the single variable. In addition, the effect of two stresses do not coincide with the effect of the mix including the two stresses (Dondero *et al.*, 2011). Several studies focused on the transcriptomic responses to the environmental stresses owing to "summer mortality" (Chaney *et al.*, 2011; Fleury *et al.*, 2012) and interactions with environmental variables that induced changes in gene expression profiles and affected the fitness of organisms (Chapman *et al.*, 2009; Chapman *et al.*, 2011; Philipp *et al.*, 2012). Beyond that, the hydrothermal vent mussel (*B. azoricus*) itself thrives in a condition with the darkness, extreme cold, high pressure and rich in methane and sulfides (Egas *et al.*, 2012) which is full of multi-stresses.

Summer mortality causes a serious influence to the aquaculture of bivalves, particularly oysters. Environmental stress and pathogens are known to interact and lead to summer mortality outbreaks. Differentially expressed genes associated with "immune response" biological process were significant up-regulated (Chaney *et al.*, 2011; Fleury *et al.*, 2012). Moreover, genes of oysters associated with cell death and autopahgy would suggest that at least some proportion of genes is symptomatic that underwent "summer mortality" (Chaney *et al.*, 2011). In addition to abiotic stressors, pathogen is an important factor in inducing "summer mortality". *Vibrio splendidus* is associated with summer mortality of juvenile oysters (*C. gigas*) and make juvenile oysters reduce stress-response capacities (Lacoste *et al.*, 2001). Similarly, the effects of vibrio may impair adult oyster immune defenses and cellular and immune functions that characterize the oyster capability to survive *V. splendidus* infections (De Lorgeril *et al.*, 2011). *Ostreid herpesvirus* 1 (OsHV-1) infections have been reported around the world and are associated with high mortalities of *C. gigas* in summer (Segarra *et al.*, 2010; Dégremont 2011). OsHV-1, same as *V. splendidus*, induced the oysters with significant changes in the expression of immune related genes (Renault *et al.*, 2011).

The interesting studies by Chapman *et al.* (2009, 2011) examined the transcriptomic responses of oysters to environmental stresses and land-use impacts, providing an extension of an earlier assessment of the relative gene expression patterns. Response to environmental stressors, genes encoding electron transport chain are important discriminators for the levels of metals, organic pollutants and nutrients. In addition, a suite of genes involved in the regulation of cell volume and growth, energy metabolism and stresses can also be the indicators of the environmental quality (Chapman *et al.*, 2009, 2011).

Through environmental cluster analysis, the environmental pH and the temperature were by far the leading environmental factors governing gene expression patterns with minor contributions of salinity and dissolved oxygen (Chapman *et al.*, 2011). It is noteworthy that there is a strong negative correlation, suggesting genes that are up-regulated by higher temperatures are also up-regulated by lower pH and *vice versa* (Chapman *et al.*, 2011).

The hydrothermal vent mussel survival in such extreme conditions requires unique anatomical and physiological adaptions. It has been reported that they rely on unique capabilities to detect and respond to micro associated molecular patterns such as lipopolysaccharides (LPS), lipoteichoic acids, lipoproteins, peptidoglycan (PGN) and (1→3) β-D-glucans (Bettencourt *et al.*, 2010). Under controlled hyperbaric pressure, genes of *B. azoricus* relative to heavy metal contaminants and oxidative stress differentially expressed, and the occurrence of glycosylation was changing with the elevanted hyperbaric pressure (Bettencourt *et al.*, 2011). Furthermore, *B. azoricus* survives in reducing environments rich in methane and sulfides, owing to symbiotic association with methylotrophic or methanotrophic and thiotrophic bacteria (Egas *et al.*, 2012). Enzymes involved in sulfur and methane oxidation have been found, but the molecular pathways underlying sulfur and methane oxidation within the hydrothermal vent mussel had no sufficient evidence (Egas *et al.*, 2012). At "sea-level" condition, *B. azoricus* can be used a model organism to explore more information. The main studies focused on the transcriptomic response to stress in marine bivalves are summarized in Table 1.

The transcriptomic approach

Suppression subtractive hybridization (SSH)

SSH is a PCR-based technique that allows the identification of genes that differentially expressed between two conditions (Diatchenko *et al.*, 1996). Since this technology came to being in 1984 (Lamar *et al.*, 1984), it has experienced several improvements to be more accurate and easier. Until 1996, SSH application has been maturation (Diatchenko *et al.*, 1996; Diatchenko *et al.*, 1999). This application is a powerful tool for the study of differential gene expression and the identification of

Table 1 Main studies related to transcriptomic response to stress in marine bivalve

Study	Stress	Bivalve species	Strategy
Tanguy *et al.*, 2004	*Perkinsus marinus*	*Crassostrea virginica and Crassostrea gigas*	SSH and real-time PCR on heamocytes
Brown *et al.*, 2006	benzo[a]pyrene	*Mytilus edulis*	SSH and macroarray on digestive glands of control and experimentally contaminated mussels
Dondero *et al.*, 2006	copper pollution gradient	*M. edulis*	Microarray and real-time PCR on digestive gland of experimental contaminated mussels
Venier *et al.*, 2006	metals and chemicals	*Mytilus galloprovincialis*	Microarray and real-time PCR on gills, digestive gland, muscles and mantle of naturally and experimentally contaminated mussels
Gestal *et al.*, 2007	mix of dead bacteria	*Ruditapes decussatus*	SSH on heamocytes of oysters exposed to the mix of dead bacteria
Masson *et al.*, 2007	atrazine	*M. edulis*	monitoring the gene of Dnak-type molecular chaperone
Chapman *et al.*, 2009	land-use influences	*C. virginica*	microarray on gills and hepatopancreas of oysters lived in 11 creeks along the Atlantic coast of the southeastern USA
Green *et al.*, 2009	hypoxia	*Saccostrea glomerata*	SSH on haemocytes, monitoring the expression of genes encoding anti-oxidant enzymes
Lang *et al.*, 2009	heat stress	*C. gigas*	Microarray and real-time PCR on gills of oysters exposed to high temperature
Prado-Alvarez *et al.*, 2009	*Perkinsus olseni*	*R. decussatus*	SSH on heamocytes exposed to perkinsus olseni in different time point
Wang *et al.*, 2009	*Vibrio alginolyticus*	*Pinctada fucata*	monitoring the expression of HSP 70 in the heamocytes of oysters responding to bacterial challenge
Zapata *et al.*, 2009	copper pollution gradient	*Argopecten purpuratus*	SSH and real-time PCR on post-larvae of scallop
Bettencourt *et al.*, 2010	conditions of the hydrothermal vent field	*Bathymodiolus azoricus*	RNA-seq on gills
Dondero *et al.*, 2010	nickel and chlorpyrifos	*M. galloprovincialis*	Microarry and real-time PCR on digestive gland of mussels exposed to nickel, chlorpyrifos and the mix (nickel and chlorpyrifos)
Lockwood *et al.*, 2010	osmotic stress	*M. galloprovincialis and Mytilus trossulus*	Microarray on gills of two species mussels
Sussarellu *et al.*, 2010	hypoxia	*C. gigas*	Microarray and real-time PCR on the digestive gland
Wang *et al.*, 2010	*Perkinsus marinus*	*C. virginica*	Microarray and real-time PCR on gills
Bettencourt *et al.*, 2011	hydrostatic pressure	*B. azoricus*	real-time PCR on selected genes of gills and mantles
Boutet *et al.*, 2011	environmental factors of the hydrothermal vent field	*B. azoricus*	compare differentially expressed genes in gills of two groups, one is rich in methanotrophic bacteria and the other is rich in thiotrophic bacteria using SSH and microarray
Chaney *et al.*, 2011	summer mortality	*C. gigas*	microarray on heamocytes of survival and mortal oysters
Chapman *et al.*, 2011	environmental factors	*C. virginica*	microarray on gills and hepatopancreas of oysters lived in 11 creeks along the Atlantic coast of the southeastern USA
De Lorgeril *et al.*, 2011	*Vibrio spp.*	*C. gigas*	DGE analysis on heamocytes of oysters infected by virulent and avirulent strains
Fu *et al.*, 2011	osmotic stress and bacterial challenge	*Crassostrea hongkongensis*	monitoring the expression of HSP 90 in the heamocytes of oysters under stresses
Ivanina *et al.*, 2011	cadmium and hypoxia	*C. virginica*	real-time PCR on selected genes of hepatopancreas
Lockwood *et al.*, 2011	heat stress	*M. galloprovincialis and M. trossulus*	Microarray on gills of two species mussels
Milan *et al.*, 2011	temperature and salinity	*Ruditapes philippinarum*	RNA-seq and microarry on individuals of clams exposed to quick changes of temperature of salinity

Brulle *et al.*, 2012	*Vibrio tapetis*	*R. philippinarum*	SSH and real-time PCR on heamocytes exposure to V.tapetis and V.splendidus
Egas *et al.*, 2012	endosymbionts and free-living deep-sea bacteria	*B. azoricus*	RNA-seq on gills
Fleury and Huvet, 2012	summer mortality	*C. gigas*	Microarray on muscle, gills, gonad of natural mussels in different time points
Martín-Gómez *et al.*, 2012	*Bonamia spp*	*Ostrea edulis*	SSH and real-time PCR on heamocytes exposed to Bonamia spp
Mello *et al.*, 2012	brevetoxin	*C. gigas*	real-time PCR on selected genes following heamocytes exposure to brevetoxin
Moreira *et al.*, 2012a	*V. alginolyticus*	*R. philippinarum and R. decussatus*	real-time PCR on selected genes following heamocytes exposure to vibrio
Moreira *et al.*, 2012b	*Vibrio anguillarum*	*R. philippinarum*	RNA-seq on heamocytes of clams exposed to alive and heat-inactivated vibrio
Morga *et al.*, 2012	*Bonamia spp*	*O. edulis*	SSH and real-time PCR on heamocytes
Place *et al.*, 2012	Pacific tides	*Mytilus californianus*	Microarray on gills, muscle and mantle of mussels inhabiting different vertical locations
Wang *et al.*, 2012	*V. alginolyticus*	*Pinctada martensii*	SSH and real-time PCR on heamocytes
Zhao *et al.*, 2012	osmotic stress	*C. gigas*	RNA-seq on gills of oysters exposed to different salinity gradient seawater
Choi *et al.*, 2013	hypoxia	*C. gigas*	monitoring the effects of hypoxia-inducible factor-alpha on respiratory burst activity
Guevelou *et al.*, 2013	hypoxia	*C. gigas*	monitoring the expression of AMP-activated protein kinase α
Meng *et al.*, 2013	osmotic stress	*C. gigas*	RNA-seq on gills of oysters exposed to different salinity gradient seawater
Varotto *et al.*, 2013	combined metal salts	*M. galloprovincialis*	Microarray and real-time PCR on gills

genes involved in specific biological functions, especially in organisms where genomic data are not available (Zhang *et al.*, 2001).

In molluscs, most studies aim to identify the molecular basis of the most common pathologies reviewed in Romero *et al.*, 2012. Some studies focused on several genes by SSH-cDNA libraries, such as *Hsp90*, ubiquitin gene response to osmotic stress and bacterial challenge and two catalase homologs response to bacterial infection and oxidative stress in *Crassostrea hongkongensis* (Fu *et al.*, 2011; Zhang *et al.*, 2011), a Dnak-type molecular chaperone exposed to atrazine in blue mussels (Masson *et al.*, 2007), and *Hsp70* in the haemocytes of pearl oyster responding to bacterial challenge (Wang *et al.*, 2009). Some studies investigated genes of the same categories, and mostly aimed at immune-related genes (Xu *et al.*, 2009; Martín-Gómez *et al.*, 2012; Wang *et al.*, 2012; Gestal *et al.*, 2007) and anti-oxidant genes (Green *et al.*, 2009).

With the increase of the EST databases in marine bivalves, studies containing more coverage of transcripts were undertaken, involved transciptome in response to parasites (Tanguy *et al.*, 2004; Prado-Alvarez *et al.*, 2009; Morga *et al.*, 2011), virus (Brulle *et al.*, 2012), and heavy metals (Zapata *et al.*, 2009).

The SSH technology is a quick and effective method to distinguish the genes differentially expressed by high specificity and sensitivity. It can isolate dozens of, even hundreds of genes differently expressed and is easy to operation. However, this technology bases on the hybridization, so that the genes that have no or little restriction enzyme cutting site cannot be isolated by the SSH.

Microarrays

Microarrays are on the basis of abundant genes or gene segments with known sequences. The construction of the numerous libraries has led to a significant increase in the number of ESTs in databases, which contain genes that are modulated in response to environmental stresses and can be used to design probes in microarrays. Microarrays have various applications, including the analysis of gene expression analysis and genotyping for point mutations, single nucleotide polymorphisms (SNPs), and short tandem repeats (STRs) (Heller, 2002).

In molluscs, microarrays were mostly used on the gene expression profiling of responses to environmental stresses. Lockwood *et al.* (2010, 2011) analyzed the effects of heat stress and hypo-osmotic stress between invasive and native mussels. The relative studies about gene expression profiles of the oysters exposed to hypoxia (Sussarellu *et al.*, 2010), heat (Lang *et al.*, 2009), and parasites (Wang *et al.*, 2010) were performed. Moreover, microarrays were used to analyze the interactions of multifactor in oysters (Chapman *et al.*, 2009; Manfrin *et al.*, 2010; Chaney *et al.*, 2011; Chapman *et al.*, 2011; Dondero *et al.*, 2011; Fleury *et al.*, 2012). Microarrays were also

used to decipher the effects of the environmental factors on gene expression in the deep-sea mussels (Boutet *et al.*, 2011).

Owing to the limitation that microarrays have to base on the known sequences, the studies about the marine bivalves with limited gene databases have only concentrated on several species that are important under the commercial point of view. Meanwhile, the relevant researches are so few that the costing of microarrays in marine bivalves is relatively high.

The next-generation sequencing technologies

The occurrence of the next-generation sequencing technologies is a giant leap in genomic and transcriptomic research. They make large-scale sequencing possible by high-throughput and cost-efficiency (Marguerat *et al.*, 2010). Although these powerful and rapidly evolving technologies have only been available for a couple of years, they are already making substantial contributions to our understanding of genome expression and regulation. There are three main commercially technologies, including Roche (454 Life Sciences), Illumina (Solexa Sequencing Technology), and Applied Biosystems (Life Technologies/APG).

The approach to exploit dynamic transcriptomes by the next-generation sequencing technologies termed RNA-seq. The application of these technologies is a fast and efficient approach for gene discovery and enrichment of transcriptomes in non-model organisms. Currently, transcriptomes have been sequenced for various marine bivalves. In relation to the responses to environmental stresses in molluscs, the researches concentrated in oysters to discuss the relative genes and pathways against osmotic stresses and virus infections (De Lorgeril *et al.*, 2011; Zhao *et al.*, 2012; Menge *et al.*, 2013). Furthermore, there were some literatures about the immune system of mussels (Philipp *et al.*, 2012), and clams (Moreira *et al.*, 2012).

The next-generation technologies make the sequencing of the non-model organisms possible. With the development and popularization of high-thoughput sequencing technologies, the genomes and transcriptomes of more and more marine bivalves would be sequenced.

Conclusion

The ecological status of the marine bivalves is always an important advantage to monitor the environmental quality of the intertidal zones. Along with the fast development of technologies and analysis methods, the information of the molluscs genomes increased drastically. The genomic information is the basis for understanding how the mollusks respond to environmental stresses and solving important problems in the bivalve production such as the “summer mortality”.

In this minireview, we summarized the recent studies about the transcriptomic response to stress in marine bivalve. Owing to the characteristic of bivalve’s genome and no model organism, the development of the molecular studies made a slow progress in a period. However, advanced technologies bring genome and transcriptome of bivalve new insights and progressive directions. Increasing researchers devote themselves to the mechanisms of bivalve adapted to the complex conditions at molecular level.

Nevertheless, we face several problems. Due to the decreasing cost of the next-generation technologies, we need to increase the biological repeats to increase the accuracy of the data. To date, most experiments are based on the laboratory conditions. However, environmental factors interact with each other in the nature. The researches in the future should consider the combination of imitation and the nature. Eventually, transcript levels are only a proxy for protein expression, and cannot be identical completely with protein expression because of post-translational modifications or other reasons. Comparing the results of the transcriptome and proteome, we can explain the experimental consequences more comprehensively.

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