ABSTRACT: As the research community attempts to forecast the effects of ocean acidification on marine ecosystems, a critical element is a clear understanding of the effects of ocean acidification on an individual organism’s physiology. This article explores how the use of genomics-based tools that measure gene expression—DNA microarrays and quantitative PCR—can assist in this effort and reveal aspects of how calcifying marine organisms will respond to ocean acidification. More specifically, what stands to be gained from this approach is an understanding of the direct effects of ocean acidification and whether organisms have sufficient physiological plasticity to adapt to the altered CO₂ conditions. We provide a brief overview of biomineralization processes in corals and sea urchin larvae, and then link these pathways to ways in which gene expression analysis can reveal physiological responses and mechanisms, and further, can define new testable hypotheses. In addition, we review the resources available and strategies that might be taken for each of 2 study organisms, stony corals and sea urchins. Finally, we suggest strategies for gene expression profiling in organisms that differ in availability of genomic resources.

KEY WORDS: Ocean acidification · Biomineralization · Microarrays · Calcification · Coral · Gene expression · Genomics · Sea urchin

INTRODUCTION

As the research community explores the effects of ocean acidification on marine ecosystems (Royal Society 2005, Kleyпас et al. 2006), a key link to forecasting the effects of this altered seawater chemistry is understanding the response at the organismal level. A potentially productive path for the ocean acidification research community is to leverage genomics tools (Box 1) to understand the mechanisms that might be driving altered skeleton formation in marine calcifying organisms, and in addition, to reveal whether potential compensation in the key pathways for biomineralization and other processes is possible.

Genomics approaches have been solidly integrated into the general field of ecology. Notably, transcriptomics—the measurement of all mRNAs in a biological sample, usually performed with a microarray—has recently emerged in marine ecology (Hofmann et al. 2005). Notably, since microarrays have been used to assess the physiological responses of organisms to abiotic environmental conditions (Gracey 2007), they also have the potential to highlight pathways that are changing in response to elevated CO₂.

There are many barriers to success in using microarrays or other methods to profile gene expression (e.g. quantitative PCR [qPCR]), but they generally narrow down to whether there is sufficient DNA sequence available for a particular species to support the construction and use of a microarray or the design of gene-specific primers for qPCR. Fortunately, there is significant movement in the field as more libraries and platforms are available for ecologically and economically important marine species. Additionally, in the ab-
Box 1. Quantitative PCR & microarrays. Several tools exist to quantify the expression of gene transcripts within organisms. Two promising techniques for assessing effects of ocean acidification are described.

**Quantitative PCR (qPCR).** A modification of the standard PCR in which cDNA is quantified after each round of amplification (real-time) as opposed to end-point analysis. qPCR determines the relative starting quantity of messenger RNA (mRNA) in a sample with high resolution and precision and enables the researcher to quantify relative gene expression in a cell or tissue type at a particular time. This technique focuses on a single to few genes at a time and consequently is a more targeted approach to identifying mechanisms or describing a particular physiological pathway. qPCR is also used to verify DNA microarray results for a smaller set of genes. For qPCR, sufficient sequence information is preferred to create gene-specific primers.

**Microarrays.** The power of the microarray technology is in its scale. This technique allows for the simultaneous quantification of thousands of mRNAs in a given sample and therefore enables the researcher to profile the expression of genes involved in a large number of physiological pathways in a single step. Microarrays consist of glass slides spotted with up to tens of thousands of ‘features’: short segments of single DNA sequences in high density. Using competitive hybridization of 2 alternatively labeled samples to the feature spots, microarrays measure the relative abundance of thousands of mRNAs in a ‘control’ vs ‘experimental’ sample. While microarrays lack the detailed resolution of qPCR and can be technically more challenging to implement, the capacity to profile an organism’s genome-wide response to a particular environmental condition makes this tool invaluable to ecological genomics. Below we outline the two primary microarray technologies used for gene expression analysis.

- **cDNA microarray:** For non-model systems with little to no available sequence information, it is possible to construct a library of all potentially expressed mRNA transcripts found within individuals of the target species. These cDNAs can then be spotted as ‘features’ on a microarray. cDNA microarrays are inexpensive to manufacture, but require considerable time and effort to develop the underlying cDNA library.

- **Oligonucleotide microarray:** Where sufficient sequence information exists, such as for model organisms or those with completely sequenced genomes, it is possible to select sequences from a database and simply order an array of synthesized gene-specific oligonucleotide ‘features’. While oligo arrays offer a more sensitive and reproducible microarray technology for genome-wide transcript profiling, they are considerably more expensive than cDNA arrays and may be limited in their capacity for cross-species hybridizations.

Certainly one of the obvious initial questions is: Which marine organisms best support using gene expression profiling to address cellular- and molecular-level mechanisms in ocean acidification scenarios? Another is: Which species are critical to study due to the urgency of the ocean acidification problem? Since these approaches are significantly facilitated by access to DNA sequence information, a ranking of organisms by the depth of genomic and molecular resources is perhaps one of the first steps to consider. As we see it, amongst marine organisms, there are 5 excellent candidates: the purple sea urchin *Strongylocentrotus purpuratus*, scleractinian corals, oysters, limpets and coccolithophorids. A microarray-based approach has already been used in the study of calcification in coccolithophorids (Quinn et al. 2006). For the calcifying marine invertebrates, genomics resources are available in the form of sequenced and annotated genomes (Sea Urchin Genome Sequencing Consortium 2006) or excellent microarray resources are in place (Forêt et al. 2007, Jenny et al. 2007, Desalvo et al. 2008). Other strategies are available for investigators interested in non-model but ecologically critical species. Specifically, the design of PCR primers is possible given the available sequence data in various databases (Table 1). Additionally, efforts to obtain sequence data for critical species such as pteropods in high latitude seas are underway using pyrosequencing (G. Hofmann & V. Fabry unpubl. data), and highly feasible given the increasing availability of affordable high-throughput sequencing and its proven utility in the study of ecologically important questions (Vera et al. 2008).

However, for the purpose of this article, we will focus on how to apply functional genomics to the question of the effects of ocean acidification on sea urchins, due to the availability of the data in the sequenced genome, and, secondly, for stony corals given their ecological importance in biominalization in coral reef ecosystems.

**EVIDENCE FOR THE IMPACT OF OCEAN ACIDIFICATION: WHERE TO START**

For our purposes, it would be useful to first identify the cellular mechanisms involved in biominalization,
an exercise that will highlight the types of genes that could be driving the observed changes in biogenic calcification in our 2 study organisms, sea urchins and stony corals (Fig. 1). Although numerous experimental studies have demonstrated that elevated CO2 has a sub-lethal impact on organismal, developmental and physiological features in marine calcifying organisms (Fabry et al. 2008, Guinotte & Fabry 2008, Doney et al. 2009), very little is known about the cellular-level mechanisms that alter these processes in response to elevated CO2 conditions. Additionally, since marine calcifiers have different forms of the biomineral calcium carbonate (Lowenstam & Weiner 1989), we expect the responses to vary by taxon. Thus, a taxonomically broad effort, encompassing a variety of calcifiers, will capture individual responses that can integrate to reveal impacts on ecosystem-level processes.

<table>
<thead>
<tr>
<th>Gene for:</th>
<th>Function</th>
<th>Organism</th>
<th>GenBank accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nacrein or nacrein-like proteins</td>
<td>Thought to play a role in the regulation of calcium carbonate (CaCO3) crystal formation in mollusk shells</td>
<td>Oyster</td>
<td>D83523, AB252484, AB252480</td>
</tr>
<tr>
<td>Chitin synthase (ArCS-1p)</td>
<td>Involved in chitin deposition in the mollusk shell during nacre formation</td>
<td>Pen shell</td>
<td>DQ081727</td>
</tr>
<tr>
<td>Perlustrin</td>
<td>Believed to play a role in the nucleation and/or the growth of CaCO3 crystals</td>
<td>Abalone</td>
<td>P82595</td>
</tr>
<tr>
<td>Lustrin A or lustrin</td>
<td>Control the morphology and packing of CaCO3 crystals by becoming occluded in the mineralized composite during shell formation</td>
<td>Abalone</td>
<td>AF023459, DQ298402</td>
</tr>
<tr>
<td>Perlucin</td>
<td>Believed to play a role in the nucleation and/or the growth of CaCO3 crystals</td>
<td>Abalone</td>
<td>P82596</td>
</tr>
<tr>
<td>Perlinhibin</td>
<td>Involved in the inhibition of CaCO3 crystal growth and dissolution</td>
<td>Abalone</td>
<td>P85035</td>
</tr>
<tr>
<td>Shell matrix proteins</td>
<td>Control the morphology and packing of CaCO3 crystals by becoming occluded in the mineralized composite during shell formation</td>
<td>Scallop</td>
<td>AB073617</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mussel</td>
<td>AY364453</td>
</tr>
<tr>
<td>Pearlin or pearlin-like proteins</td>
<td>Control of nucleation of the first layer of oriented calcite/aragonite in deposition of the abalone shell and flat pearl</td>
<td>Oyster</td>
<td>AB020779, AB159512, AB094512</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pen shell</td>
<td>AF145215</td>
</tr>
</tbody>
</table>

Table 1. Candidate genes in marine calcifying organisms that currently lack a sequenced genome

Sea urchins

Due to its status as a model organism for development, the purple sea urchin has emerged as the marine invertebrate with the deepest genomic resources. Combined with a developed view of how biomineralization occurs in sea urchin embryos (Wilt 2002), the opportunity to use genomics approaches to explore the expression of genes involved in biomineralization are rich (Livingston et al. 2006). When considering how to begin these studies, we have identified a suite of genes that, if targeted, can reveal considerable detail into how ocean acidification and elevated CO2 will impact biomineralization and skeleton formation in larval and adult sea urchins (A. Todgham & G. Hofmann unpubl. data). These 3 classes are (1) genes for proteins in the organic matrix, (2) genes for transporters in membranes, and (3) genes coding for carbonic anhydrase, an enzyme that drives CO2 elimination in cells (Fig. 1).

If, in this first-cut analysis, we focus on the effect of CO2 on the process of spicule formation in the sea urchin larvae, we would examine genes that are involved in biomineralization during skeletogenesis. In sea urchins, the spicule is formed by primary mesenchyme cells (PMCs) where the PMCs act as a cytoplasmic sheath around the forming spicule (Fig. 1). Gene expression in the PMCs is thought to be involved in calcium transport where calcium is transported from the external seawater, modified in the PMC cytoplasm, and then moved via exocytosis into the extracellular space around the forming spicule. In addition to genes involved in calcium transport to form calcite, there are proteins that facilitate precipitation of calcium in the spicule and there are also 45 proteins that have been identified in association with the spicule. Although the roles of all these proteins are not known, some of them are well known, e.g. SM30 is embedded in the mineral phase of the spicule and SM30 and SM50 have high expression rates at the growing nascent tips of larval spicules (Wilt 2002). Should sea urchin larvae be able to compensate for the impact of CO2 on biomineral-
ization, one might predict that there would be changes in the expression of these genes. Notably, recent studies have shown sub-lethal effects of CO2 on skeleton formation in sea urchins (Kurihara & Shirayama 2004), and gene expression studies indicate that expression patterns of spicule matrix proteins change with CO2 levels (A. Todgham & G. Hofmann unpubl. data).

**Corals**

Because a fully sequenced genome is not available for a species of stony coral, the resources are not as advanced as they are for sea urchins. However, there are DNA sequence data available for numerous species (reviewed in Forêt et al. 2007), microarray and qPCR studies are very achievable (de Boer et al. 2007, Schwarz et al. 2008, Mayfield et al. 2009), and this effort could easily be turned to questions of the impact of ocean acidification.

For corals, skeleton formation requires the transport of calcium and dissolved inorganic carbon (DIC) from seawater to the site of calcification at the epithelium of the calicoblastic cells (Fig. 1) of a coral polyp to form aragonite, a calcium carbonate mineral that makes up the skeleton in combination with the organic matrix (reviewed in Allemand et al. 2004). Although our ‘gene targeting’ approach is less clear-cut due to the complexities of coral skeletogenesis, ocean acidification impacts on biogenic calcification in corals can be examined by looking at active processes that are driven by a protein or a transport mechanism (Fig. 1).

In terms of calcium transport, early work indicated that calcium is delivered to the site of calcification by transcellular transport (reviewed in Gattuso et al. 1999, Cohen & McConnaughey 2003). Recent research supports these earlier studies and measured intracellular gradients of calcium that suggested the active, transcellular transport of calcium (Marshall et al. 2007). Calcium channels have been found in the calicoblastic epithelium and a goal would be to target the expression of these genes (Zoccola et al. 1999).

For the carbon source, benchmark research first showed that the carbonate in the skeleton can originate from 2 carbon sources, either from metabolic CO2 or from soluble carbonate in external seawater. More recent research has focused on the source of carbon for coral skeleton formation and has pointed towards cel-
lular processes of interest. For example, the role of carbonic anhydrase has recently been the focus of biochemical research and this enzyme activity is found in tissues and in the organic matrix of an azooxanthellate coral (S. Tambutte et al. 2007). In addition, immunochemical methods have shown that calicoblastic cells are secreting components of the organic matrix (Pverel et al. 2005, 2007) and some of these matrix proteins have been cloned (Fukuda et al. 2003). Taken together, these accumulating experimental observations, and studies further describing the tissue–skeleton interface, argue for an active role of calicoblastic cells in the physiological process that controls calcification of the coral skeleton (E. Tambutte et al. 2007), and that, for example, carbonic anhydrase expression would be a good target of study. Most importantly, if more genes are explored in this endeavor, it will be possible to get a physiological fingerprint of the response of corals to ocean acidification and have a more comprehensive view of calcification. This endeavor is underway as more genomic resources for corals become available, a situation that will lead to clearer understanding of the skeletonogenesis in corals in general, and then how this process will respond to ocean acidification at the molecular and cellular level.

GLOBAL PHYSIOLOGICAL RESPONSE TO OCEAN ACIDIFICATION

It should not be forgotten that a transcriptomics approach also affords the investigator a view of many metabolic processes, not just the activity of those genes involved in biomineralization. In many ways, this ‘discovery’ aspect of the genomics approach supplies a platform on which future hypotheses, and a search for mechanism, can be built. Most importantly, this perspective will provide a more complete understanding of whether marine calcifiers have the physiological plasticity to compensate for the effects of ocean acidification and continue to build skeletons under future CO2 conditions. Microarray expression profiling has been used in numerous studies on non-model organisms to reveal patterns of physiological response to environmental factors (Gracey 2007) and this approach has revealed important transcriptional responses to environmental stressors in non-model marine organisms (Podrabsky & Somero 2004, de la Vega et al. 2007, Kassahn et al. 2007, Kultz et al. 2007, Teranishi & Stillman 2007, Place et al. 2008). In an ocean acidification scenario, one notable organismal function that would be perturbed is acid/base balance (Pörtner et al. 2005). Organismal studies have shown an effect of CO2 on acid/base balance in calcifiers such as sea urchins (Miles et al. 2007). Thus, the study of acid/base balance in marine organisms is an example of how gene expression profiling might reveal genes that are changing, or steps in metabolic pathways that are being altered, in response to a changing abiotic environment. Finally, recent studies on coral larvae have identified genes that are involved in the cross-talk between the algal symbionts and the invertebrate host (de Boer et al. 2007). Such studies could be extended to assess the effects of ocean acidification on the algal–coral symbioses from a more global perspective, i.e. whether the association of coral with their Symbiodinium sp. symbionts will change as a function of different seawater chemistry.

SUMMARY

The application of genomics approaches to the question of the impact of ocean acidification will likely develop as fast as the resources become available. With the increase in the use of cross-species hybridizations (Buckley 2007), there is the opportunity to extend these resources without making gene chips for every species under study. Overall, gene expression profiling gives us a powerful tool to begin to understand how the physiology of marine calcifying organisms is likely to change in the face of a more acidic ocean. Targeted studies of individual species are significant in that each calcifier’s response will vary and thus the ecosystem-level impact will be transduced through the physiology of key species. Although gene expression is but one technique (there are other approaches in systems biology, e.g. proteomics or metabolomics), there is great potential to learn about the complexity of the compensatory responses in calcification and other metabolic pathways under ocean acidification conditions. Additionally, transcriptome profiling and its ability to reveal subtle, complex patterns will be a powerful approach to tease apart interacting stressors such as the synergistic effects of ocean acidification and warming, the ‘double jeopardy’ scenario within global climate change. Overall, the use of functional genomics will contribute to 2 important unknowns in the effort to forecast the effects of ocean acidification on marine ecosystems: (1) What are the basic organismal responses to the predicted levels of CO2? and (2) Will marine calcifying organisms have sufficient plasticity to build skeletons in a high-CO2 world?

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