We have characterized two major components of the ceramide biosynthesis pathway – *serine palmitoyltransferase* (*sptlc*) and *acid sphingomyelinase* (*SMAse*) – in *Crassostrea gigas*, as shown by laboratory and *in silico* analysis. The discovery of these genes in *C. gigas* adds to a growing group of apoptosis-related genes in molluscs (Lee et al. 2010; Zhang et al. 2011). Unlike the previously described genes in molluscs, however, *sptlc* and *SMAse* are directly responsible for generation of ceramide in de novo and catabolic synthesis, respectively. Further *in silico* analysis of the *C. gigas* transcriptome also shows that there are a number of other genes in this same ceramide biosynthesis pathway that have not yet been fully characterized. Full characterization of the ceramide pathway will increase our understanding of how this commercially and environmentally important species interacts with its environment and the stressors it encounters therein.

The taxonomic universality and imortance of the ceramide pathway and apoptosis in regulating an organism’s response to environmental changes is becoming increasingly acknowledged. Ceramide is an important component of the cyclooxygenase and prostanglandin synthase pathway, modulating secretion of prostaglandin E2 and activating transcription of cyclooxygenase (Hannun 1994). TNFa works through ceramide in both apoptosis and inflammation (Verheij et al. 1996). Stress-induced apoptosis is signaled by ceramide upon exposure to ionizing radiation, hydrogen peroxide, UV radiation, and heat shock (Verheij et al. 1996). Catabolic generation of ceramide is an important immediate and efficient response to radiation (Haimovitz-Friedman 1994). Abrupt changes in salinity can trigger shifts in ceramide metabolism in sea bass, most likely in an effort to mediate cellular rearrangement after osmotic shock (El Babili et al. 1996).

*C. gigas* has ESTs that correspond with high homology to a majority of the elements in the ceramide pathway (Figure 5). The genes not identified in this study or in Zhang et al. (in press) were discovered through *in silico* analysis of *C. gigas* 454 contigs of larval sequences and through BLAST searches based on homology. We identified almost all the enzymes necessary for de novo ceramide synthesis, assuring the conservation of this segment of the pathway in Pacific oysters. We also identified a number of enzymes responsible for transformation of ceramide into other lipid products: ceramide kinase (leads to ceramide 1-P), ceramide synthase (sphingosine), glucosylceramide synthase (glucosylceramide) and sphingomyelin synthase (sphingomyelin). Also identified were a variety of caspases, TNF superfamily receptors, RIP and FADD subunits of the TNFa receptor. Even though a few components of the overall ceramide pathway were not found in our searches, they most likely exist within *C. gigas*, but have either not been sequenced or the inherent poor quality and incomplete sequences of ESTs make them unidentifiable as homologs. The discovery of the enzymes that regulate generation and transformation of ceramide are of particular importance in understanding *C. gigas* response to its environment since these are the genes that will respond and adjust to changes. Since some of these genes come from a larval transcriptome, we now have evidence of the presence of this pathway in early stages of development of *C. gigas*. Apoptosis is an instrumental process during development as cells and tissues rearrange and differentiate. Genes in the ceramide pathway are essential to development in a number of vertebrates (e.g. Eliyahu et al. 2007; Yabu et al. 2001) and the presence of these genes in the oyster provide evidence of their importance in invertebrate development as well. Below we discuss in detail the two genes characterized in this study and their potential roles in oyster physiology.

Serine palmitoyltransferase catalyzes the first step of ceramide de novo biosynthesis: the condensation of serine and palmitoyl-CoA to 3-ketosphinganine (reviewed in Hannun 1994; Fig. 5). There are two forms of human Sptlc – Sptlc 1 and 2. Sptlc2 has a conserved motif that binds pyridoxal phosphate (PLP) (Hanada 2003), but *C. gigas* has an asparagine in that position (residue 311 on our alignment), which is homologous to the *H. sapiens* Sptlc1 form of the enzyme. The basic function of Sptlc is conserved across taxa and is instrumental in determining levels of ceramide and other sphingolipid intermediates (Hanada 2003). The expression of Sptlc mRNA and protein in vertebrates is sensitive to a number of biological processes, including stage of development, external stress stimuli, and apoptotic stimuli that activate the enzyme post-transcriptionally (Hanada 2003). These enzymatic functions are probably conserved in *C. gigas* considering the high degree of homology between the oyster and human amino acid sequences: 30.9% pairwise identity (over 259 residues). The *C. gigas* sequence shares 51.7% identity over 265 amino acid residues with the *S. purpuratus* Sptlc1, indicative of a very high degree of homology between these taxa. The *S. purpuratus* protein has the conserved transmembrane domain that is also seen in vertebrates (Hanada 2003), suggesting that *C. gigas* probably possesses it as well although we do not have the coverage to tell definitively.

Sphingomyelinases play a complementary role to Sptlc by catalyzing the catabolic generation of ceramide from sphingomyelin (reviewed in Ballou et al. 1996). There are two isoforms of sphingomyelinases: neutral SMase operates in the plasma membrane and acid SMase carries out its functions in the lysosome or endosome (reviewed in Kolesnick 1998). Like Sptlc, SMases are stimulated by external stimuli and their effects are seen within seconds or minutes (Kolesnick 1998). The two isoforms have distinct biological functions and acid SMase is most likely involved in TNF-mediated apoptosis (Kolesnick 1998). Acid SMase is also instrumental in vertebrate development – loss of its activity in early development of mice results in apoptotic death (Eliyahu et al. 2007). *C. gigas* acid sphingomyelinase shares fewer amino acids in common with its vertebrate counterparts, with only 21.5% identity with *H. sapiens* over 291 amino acids. *S. purpuratus* and *C. gigas* are identical for 28.1% of their amino acid residues, whereas oysters share 32.5% of their amino acids with the hemichordate *S. kowlevskii*. *S. purpuratus* and *H. sapiens* share 36.9% identity over 452 sites. This intriguing pattern indicates that *C. gigas* may possess a more basal form of SMase than other invertebrates.

Recent studies have found that apoptosis regulation is prevalent in invertebrates and probably instrumental in response to environmental stress. Zhang et al. (2010) found 52 apoptosis-related genes in *Bombyx mori*, including 5 members of the caspase family and 2 in the TNF superfamily. The disk abalone, *Haliotis discus discus*, has constitutive expression of caspase-8, the highest levels being in the gill (Lee et al. 2010). There is immediate upregulation of this gene in gill tissue and hemocytes following bacterial and viral challenge (Lee et al. 2010). In addition to the two genes discussed in this study, four more apoptotic genes were recently described in *C. gigas*: Fas-associated protein with death domain (FADD), inhibitor of apoptosis (IAP), and initiator and effector caspases (Zhang et al. in press). Similarly to the disk abalone and the expression patterns seen for *C. gigas* *sptlc*, both caspases showed the highest transcript levels in gill and mantle tissue and the lowest in the gonad and digestive gland (Zhang et al. in press). FADD and IAP had the highest mRNA transcript levels in the hemolymph (Zhang et al. in press). Overall, Zhang et al. found that there was a relatively high level of complexity in the *C. gigas* apoptosis components that they described, between those of ecdysozoa and deuterostomes, indicating gene loss in the former or possible expansion in the latter. In accordance with our findings and with Lee et al.’s (2010), FADD, IAP, and the caspases showed increased expression immediately following challenge with a *Vibrio* bacteria until expression peaked at 12 hours post-challenge (Zhang et al. 2011). Since vertebrate apoptosis and ceramide synthesis are sensitive to a wide range of environmental stressors beyond disease, the significant role in immunity played by the ceramide pathway is probably a more universal response to perceived environmental stress.