**Characterizing DNA methylation patterns in the Pacific oyster (*Crassostrea gigas)***

**Abstract Notes**

DNA methylation is an epigenetic mechanism that cells use to control gene expression. Epigenetic variation in DNA methylation provides an evolutionarily and ecologically important source of phenotypic variation among organisms. Epigenetic changes represent an important process by which environmental factors influence an organism’s gene expression, both within an individual and across generations.

This research increases our understanding of the evolution of DNA methylation in invertebrates, using *C. gigas* as a model aquatic organism. Our study represents the first attempt to characterize genome-wide methylation of *C. gigas.*

We have determined DNA methylation patterns in *C. gigas* sperm cells using whole-genome bisulfite sequencing. This approach was used to determine genome-wide methylation patterns and assess how specific DNA methylation patterns influence transcriptional activity. Our findings provide a comprehensive characterization of the mechanisms associated with DNA methylation.

**Introduction**

Epigenetics describes DNA modifications that change gene expression without altering the underlying nucleotide sequence. This includes DNA modifications by methyl groups and histones. DNA methylation in particular is established to change gene expression as an adaptive mechanism in response to environmental changes. DNA methylation involves the addition of a methyl group to a cytosine pyrimidine ring or an adenine purine ring. These methyl groups project into the major groove of DNA and effectively inhibit transcription (Baylin, 2005). The distribution and function of DNA methylation is diverse both within and among phylogenetic groups. DNA methylation has been well studied in vertebrates and mammals, however it remains understudied in invertebrates with limited information on DNA methylation, particularly in mollusks.

Although the genome may appear stable, it is constantly reacting to the environment by incorporating stable changes in the DNA sequence over generations. Epigenetic modifications of the genome provide a mechanism that allows the stable propagation of gene activity states from one generation of cells to the next (Jaenisch and Bird 2003). Furthermore, a growing body of literature suggests the role of epigenetic factors in the complex interplay between genes and the environment (Ling and Groop 2009). Environmental factors have been known to trigger epigenetic changes (Anway et al. 2005**)**. The epigenome therefore serves as an interface between the dynamic environment and the inherited static genome (Szyf 2007). The regulation and characteristics of DNA methylation remain enigmatic although it has been implicated in a range of processes like genomic integrity, X-chromosome inactivation, genomic imprinting, transposon silencing and diseases including cancer and cardiovascular disorders (Sati 2012). Therefore, it is imperative to resolve DNA methylation distribution patterns at the genomic level in order to understand these processes.

The amount and location of methylation occurring in organisms is extremely diverse and variable among species. It has been previously proposed that limited methylation may contribute to increased phenotypic plasticity in highly fluctuating environments (Roberts and Gavery 2012). Methylation most often occurs as part of C-G nucleotide pairs. In mammals, 70-80% of CpG nucleotides are methylated (Bird, 1980), which is a pattern referred to as global methylation. Invertebrates, however, display a wide range of DNA methylation, from very limited methylation in *Drosophilia melanogaster* (Gowher 2000) to a fractional or mosaic pattern of methylation in the sea urchin *Strongylocentrotus purpuratus* (Bird, 1979)*.* Considering the significant diversity of DNA methylation across taxa, it is likely that methylated areas have different functions between organisms. The variety in organismal DNA methylation patterns furthermore highlights the possibility that different methylation distributions may reflect different functions. As methylated cytosines are susceptible to spontaneous conversion into thymines through chemical deamination, they tend to be generally underrepresented in the genome and they are often grouped in dense patches termed CpG islands (Bird, 1980). CpG islands are frequently associated with promoters, and the regulation of promoter methylation has been shown to affect the expression of the corresponding transcripts (Krueger, 2012).

The Pacific oyster *Crassostrea gigas* is exposed to environmental variations including changes in temperature, pH, and oxygen levels. *C. gigas* lives at a range of tidal heights and routinely encounters large seasonal fluctuations in temperatures (Hamdoun et al. 2009). *C. gigas* spends time both above and underwater, so understanding mechanisms of coping with a static environment would prove to be useful information. Due to the physical characteristics of water, the aquatic environment can be extremely stressful to its inhabitants. In general, the high specific heat and thermal conductivity of water ensures that the majority of aquatic organisms will have body temperatures equivalent to their surroundings (Feder and Hofmann, 1999). Withstanding such variable environmental conditions requires implementation of synchronized responses to cellular stress. However, we lack a full understanding of the mechanisms underlying this response in oysters, or whether these mechanisms are conserved between oyster species (Gavery and Roberts 2010).

*C. gigas* is an excellent model for studying environmental effects on genetic modifications because its life history characteristics make it a key bioindicator species and because genomic resources for this species have recently become available with the release of the Pacific oyster genome (Zhang et al. 2012). *C. gigas* is also one of the few invertebrates with traits established to vary in response to the environment, which suggests epigenetic modifications. Elucidating the functional significance of DNA methylation in *C. gigas* may prove valuable for understanding the effects of environmental stress in aquatic organisms (Gavery and Roberts, 2010). Due to the limited knowledge of methylation in invertebrate and particularly aquatic species, it is important to examine genome-wide methylation and the processes associated with methylated areas in the *C. gigas* genome. *C. gigas* was used as a model organism to provide an in-depth examination of genome-wide DNA methylation. Our results demonstrate a relationship between methylation location and gene function, suggesting that DNA methylation performs important regulatory functions in *C. gigas.*

**References**

Anway, Matthew D.; Cupp, Andrea S.; Uzumcu, Mehmet; and Skinner, Michael K. 2005. Epigenetic Transgenerational Actions of Endocrine Disruptors and Male Fertility. Science. Vol. 308 no. 5727: 1466-1469.

Baylin, Stephen B. 2005. DNA methylation and gene silencing in cancer. Nature Clinical Practice Oncology, 2: S4-S11.

Bird, Adrian, Taggart, MH, Smith BA. 1979. Methylated and unmethylated DNA compartments in the sea urchin genome. *Cell*. 17: 889-901.

Bird, Adrian. 1980. DNA methylation and the frequency of CpG in animal DNA. Nucleic Acids Resources. 8: 1499-1504.

Bird, Adrian, Taggart, MH, Smith BA. 1980. Methylated and unmethylated DNA compartments in the sea urchin genome. *Cell*. 17: 889-901.

Bird, Adrian. 1980. DNA methylation and the frequency of CpG in animal DNA. Nucleic Acids Resources. 8: 1499-1504.

Feder, Martin E. and Hofmann, Gretchen E. 1999. Heat-Shock Proteins, Molecular Chaperones, and the Stress Response: Evolutionary and Ecological Physiology. Annual Review of Physiology. 61: 243-82.

Gavery, Mackenzie R. and Roberts, Steven B. 2010. DNA methylation patterns provide insight into epigenetic regulation in the Pacific Oyster (*Crassostrea gigas).* BMC Genomics, 11: 483.

Gowher H.; Leismann, O.; Jeltsch A. 2000. DNA of *Drosophila melanogaster* contains 5-methylcytosine. *The European Molecular Biology Organization Journal.* 19: 6918-6923.

Hamdoun, Amro M.; Cheney, Daniel P.; and Cherr, Gary N. 2009. Phenotypic Plasticity of HSP70 and HSP70 Gene Expression in the Pacific Oyster (Crassostrea gigas): Implications for Thermal Limits and Induction of Thermal Tolerance. Biological Bulletin, 205:160-169.

Jaenisch, Rudolf and Bird, Adrian. 2003. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nature Genetics: 33: 245-254.

Ling, Charlotte and Groop, Leif. 2009. Epigenetics: A Molecular Link Between Environmental Factors and Type 2 Diabetes. Diabetes, Volume 58.

Roberts, Steven B. and Gavery, Mackenzie R. 2012. Is there a relationship between DNA methylation and phenotypic plasticity in invertebrates? Frontiers in Physiology, 2: 116.

Sati, Satish; Tanwar, Vinay S.; Kumar K. Anand; Patowary, Ashok; Jain, Vaibhav; Ghosh, Sourav; Ahmad, Shadab; Singh, Meghna; Reddy, S. Umakar; Chandak, Giriraj R; Raghunath, Manchala; Sivasubbu, Sridhar; Chakraborty, Kausik; Scaria, Vinod; Sengupta, Shantanu. 2012. High Resolution Methylome Map of Rat Indicates Role of Intragenic DNA Methylation in Identification of Coding Regions. PLoS ONE 7(2).

Szyf, Moshe. 2007. Review: The Dynamic Epigenome and its Implications in Toxicology. Toxicological Sciences, 100(1): 7-23.

Zhang, Guofan; Fang, Xiaodong; Guo, Ximing; Li, Li; Luo, Ruibang; Xu, Fet et al. 2012. The oyster genome reveals stress adaptation and complexity of shell formation. Nature. 490: 49-54.