Are Circadian cycles the dominant proteome rhythm in the intertidal mussel *Mytilus californianus*?

Kristina Koster[1,3], Cory Elowe[1,2,3], and Lars Tomanek[1,2,3]

1) Biological Sciences Department, California Polytechnic State University, San Luis Obispo, CA
2) Center for Coastal Marine Sciences 3) Environmental Proteomics Laboratory

OBJECTIVES

Mytilus californianus, also known as the California mussel, is a marine bivalve that is abundant along the West coast from Alaska to southern Baja California. They mainly reside in the upper-middle intertidal zone and cling to pier pilings and surf-exposed rocks. They create multi-layered beds, which form a habitat for algae and many species of invertebrates.

Intertidal mussels live in a naturally dynamic environment. It has previously been reported (Connor and Gracey, 2011) that the 24-hour circadian (day to night) rhythm of the intertidal mussel *Mytilus californianus* is primarily responsible for its rhythmic gene expression, as opposed to the 12.4-hour tidal cycles. Because tidal cycles challenge intertidal mussels through heat stress, salinity stress, hypoxia, and food availability, the dominance of the circadian cycle is surprising. However, transcriptomics may fail to detect up to half of the variation in the proteins that comprise the final functional phenotype of the organism. Using two-dimensional gel electrophoresis and mass spectrometry, we aimed to identify whether the proteome—the protein expression—of this organism also followed the same circadian rhythmic expression as its transcriptome.

EXPERIMENT

We used a proteomic approach to assess the cellular processes during a simulated 48 hour circa-tidal and circadian rhythm using a tidal simulator and alternating light and dark cycles.

Samples were collected from Hazard Reef in Montaña de Oro in Los Osos, CA. We used gill tissue to separate proteins with 2D gel electrophoresis and identify protein expression patterns (two-way ANOVA, $p<0.02$; followed by hierarchical clustering). Proteins of interest were identified with tandem mass spectrometry.

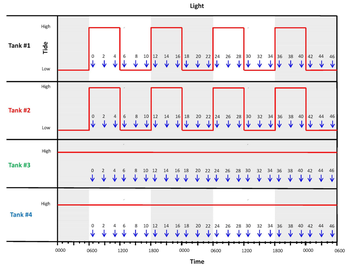


Figure 1: Experimental design for 48 hour circa-tidal and circadian rhythm using a tidal simulator and alternating light and dark cycles following four weeks of acclimation to each treatment, respectively. For the purposes of the STAR program and this poster, all samples from tanks 3 and 4 were compared for time point 0.

RESULTS

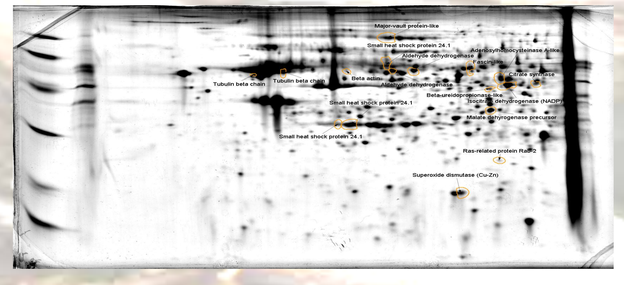


Figure 2: 2 dimensional gel image matching. Each of the six samples for tanks 3 & 4 at time point 0 were run through 2 dimensional gel electrophoresis and matched using Delta2D to form a single, fused gel image. Each spot was then analyzed to determine which protein spots were upregulated or downregulated, as compared to mean spot density of the fused gel image.

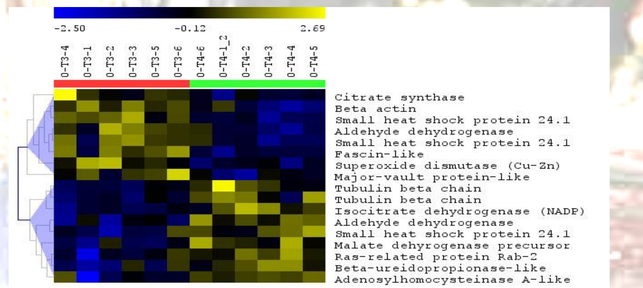
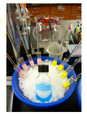


Figure 3: Example expression profiles for significant protein spots. Significance was determined using a one-way ANOVA based on 1000 permutations with a p-value <0.02, and hierarchical clustering analysis. Relative expression levels are displayed on the Y-axis. The red bar represents samples from treatment tank 3, the green bar represents samples from treatment tank 4.

PROTEOMIC WORKFLOW*

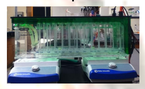
SAMPLE PREPARATION



7M Urea, 2 M Thiourea-based buffer
Protein precipitation

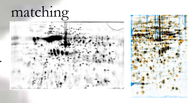
ISO-ELECTRIC FOCUSING AND SDS-PAGE
1st and 2nd Dimension

+



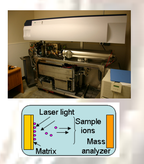
11 cm pH 4-7 IPG strip
(500 µg of protein)

ANALYSIS
Delta 2D



2D gel image matching & quantification
Stats: 2-way ANOVA, clustering

MASS SPECTROMETRY



Trypsin digestion, Matrix-assisted laser disorption ionization (MALDI) tandem time-of-flight (ToF-ToF)

* following protocols published in (Tomanek & Zuzow, 2010; Tomanek et al. 2011; Serafini et al. 2011; Fields et al. 2012)

DISCUSSION

- **Using the proteomic approach, we have been able to observe thousands of protein spots at one time and create protein expression profiles for each treatment group. This enhances our ability to identify protein candidates involved in the circadian and circa-tidal rhythms.**
- **The proteomic workflow has been successfully employed to examine the impact of circadian versus circa-tidal rhythms. A total of 448 protein spots were analyzed using one-way ANOVA to identify spots that changed significantly between the two treatments.**
- **Using heat maps and hierarchical clustering, significant spots were grouped into two clusters based on similar expression profiles. 32 proteins were significant, however only 17 of them were identified.**
- **Some of the identified proteins may support circadian rhythmic protein expression, however further examination of all the samles at each time point across treatment groups will need to be done to verify this hypothesis.**

BIBLIOGRAPHY

Connor, K. M., and A. Y. Gracey. "Circadian Cycles Are the Dominant Transcriptional Rhythm in the Intertidal Mussel Mytilus Californianus." *Proceedings of the National Academy of Sciences* 108.38 (2011): 16110-6115. Web.

ACKNOWLEDGEMENTS

- This project was supported by a grant to the STAR program from the Howard Hughes Medical Institute.
- Thanks to the Environmental Proteomics Laboratory members for their help and support.