ANALYSIS OF DIURNAL CHANGES IN PROTEIN ABUNDANCE IN THE DINOFLAGELLATE, *Karenia brevis*

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The red tide dinoflagellate, *Karenia brevis*, is a eukaryotic protist responsible for harmful algal blooms (HABs) that occur annually in the Gulf of Mexico. The mechanisms controlling gene expression in this dinoflagellate are therefore of interest. Our laboratory has recently demonstrated post-transcriptional regulation of gene expression in several key processes generally under transcriptional control in eukaryotes, including the cell cycle and acute stress responses. We have also identified in *K. brevis* an unusual RNA trans-splicing mechanism that suggests the presence of constitutive transcription of mRNA as found in trypanosomes. This suggests that gene expression may be regulated largely at the translational or post-translational level. In order to assess whether translation plays the major role in regulation of gene expression in *K. brevis*, we have employed proteomic analysis of global protein expression over the diurnal cycle, a primary regulator of physiology in photosynthetic organisms. In the current study, we collected cells from triplicate cultures at four points over the diurnal cycle, circadian times (CT) 2, 8, 14 and 22 (where CT0 is the time lights come on). Total protein was extracted using Trizol reagent, followed by two-dimensional gel electrophoresis. Samples (30 *u*g) were focused using 4-7 isoelectric focusing strips and analyzed by 2-dimensional electrophoresis on 8-16% gels, and stained using Sypro Ruby. PDQuest software was used for spot matching and intensity analysis. Approximately 17% of proteins examined in this study appear to be significantly changing (>2-fold) over the cycle. Several different patterns of expression were apparent, with the most frequent pattern being downregulation at night. Attempts to identify some of these changing proteins using mass spectrometry are underway. Once proteins are identified, protein abundances will be compared with their transcript levels previously determined by microarray analysis.

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