Post-transcriptional Regulation of the Cell Cycle in the Red Tide Dinoflagellate, *Karenia brevis*

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The dinoflagellate, *Karenia brevis*, produces harmful algal blooms in the Gulf of Mexico that cause extensive marine animal mortalities and human illness nearly annually. The molecular mechanisms controlling cell cycle entry in this dinoflagellate are important because bloom development occurs through vegetative cell division. Microarray and qPCR studies have demonstrated that, unlike typical eukaryotes, dinoflagellate cell cycle genes are not regulated at the transcriptional level, including genes that code for replication fork proteins, typically activated by the E2F transcription factor at the G1/S transition. Post-transcriptional control of these genes is also suggested by the presence of a trans-spliced leader sequence on their transcripts. Sequence analysis and protein modeling were used to develop custom antibodies for Western blotting to investigate the abundance of replication fork proteins over the cell cycle and whether they are regulated at the translational or post-translational level. The *K. brevis* replication fork proteins, PCNA, RFC, RPA and RnR2 were shown to change over the cell cycle with highest expression at S-phase, suggesting translational control. PCNA also appears to be modified post-translationally, either by ubiquitin or SUMO concurrent with S-phase. Immunolocalization of PCNA showed that it is present in the nucleus throughout the cell cycle in cells actively traversing the cell cycle. However, PCNA showed a pattern of nuclear location that changes between a chromatin bound form and a pool that is peripheral. These results lead us to propose a novel mechanism of translational control of cell cycle entry as opposed to transcriptional control which is seen in most eukaryotes.

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