Post-transcriptional regulation of the DNA Replication Fork Protein, PCNA, in the Florida Red Tide dinoflagellate, *Karenia brevis*

Stephanie A. Brunelle[1,2] and Frances M. Van Dolah[2,1]

[1]*Marine Biomedicine and Environmental Sciences, Medical University of South Carolina, Charleston, SC*
[2]*Center for Coastal Environmental Health and Biomolecular Research, National Oceanic and Atmospheric Administration, Charleston, SC*

The dinoflagellate, *Karenia brevis*, is responsible for harmful algal blooms in the Gulf of Mexico that cause extensive marine mortalities and human illness on a nearly annual basis. The molecular mechanisms controlling the cell cycle 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 replication fork proteins that are typically activated by the E2F transcription factor at the restriction point, which regulates entry into S-phase. Post-transcriptional control of these genes is further suggested by the presence of a trans-spliced leader sequence on their transcripts. The current study investigated whether the expression of the replication fork protein, PCNA, is regulated at the translational or post-translational level. Immunolocalization using a peptide antibody developed for *K. brevis* PCNA showed that PCNA is constitutively present in the nucleus throughout the cell cycle. However, its distribution within the nucleus changes, with prominent staining of chromatin-bound PCNA during S-phase, whereas it is present in a peripheral pool during the rest of the cell cycle. A similar pattern is observed in the trypanosome, a protist that also possesses a spliced leader sequence and controls gene expression at the post-transcriptional level. Western blot data similarly show that PCNA is present during the entire cell cycle, although it appears to be more highly expressed during S-phase than G1. However, there appears to be an increase in the size of PCNA from 28 kDa during G1 to a ~37 kDa band dominating during S-phase, and both forms present during G2 and M. We hypothesize that this change in size may be due to sumoylation of the protein concurrent with its chromatin association, as has been observed in yeast. We have identified a ~9kDa sequence in our *K. brevis* cDNA library with homology to SUMO (small ubiquitin like modifier), indicating that dinoflagellates possess the SUMO mechanism for post-translational protein modification. Furthermore, immunoprecipitated *K. brevis* PCNA probed with an antibody to SUMO revealed an immunoreactive band at ~37 kDa. Together, our results suggest that the expression and activity of PCNA is controlled at both the translational and post-translational levels in this dinoflagellate, in the absence of transcriptional regulation.

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