Polyketide synthases in *Karenia brevis*: characterization and expression of proteins involved in toxin biosynthesis in the Florida red tide dinoflagellate <u>Emily A. Monroe<sup>1,2</sup></u>, Zhihong Wang<sup>2</sup>, Richard K. Pierce<sup>3</sup>, and Frances M. Van Dolah<sup>1,2</sup>

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Karenia brevis is the causative organism of Florida red tides that are responsible for detrimental effects on the environment and on human health through the production of brevetoxins. Brevetoxin biosynthesis is proposed to be mediated by polyketide synthase enzymes (PKS). We have recently demonstrated novel and unexpected type I PKS transcript structures in K. brevis that encode for single catalytic domains. The absence of differential expression of PKS's in microarray analyses and the presence of a spliced leader sequence led us to hypothesize that these transcripts are controlled post-transcriptionally. In this study, we employed peptide polyclonal antibodies to K. brevis PKS sequences to analyze protein abundance in a low toxin-producing K. brevis (Wilson) sub-strain compared to K. brevis (Wilson) cultures that produce 8 pg/cell intracellular brevetoxins. During log phase of growth, RNA, protein, and brevetoxins were isolated from the low toxin-producing cultures and control cultures. Brevetoxins were not detected in any of the low toxin-producing cultures by LC/MS (detection limit <0.1 pg/cell). Using our K. brevis microarray and qPCR to examine PKS transcript levels, PKS transcript expression in the low toxin-producing cultures was not significantly different from control cultures. However, abundance of certain PKS proteins was depressed 40-60% in the low toxin-producing cultures, suggesting these proteins may be involved in toxin biosynthesis. This is the first evidence relating expression of a gene or protein to toxin biosynthesis in a dinoflagellate. Identification of the molecular basis of toxin biosynthesis is a critical step in understanding regulation of toxin biosynthesis.

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