

GENE EXPRESSION PROFILING AROUND THE CLOCK IN THE ANTARCTIC KRILL (*EUPHAUSIA SUPERBA*)

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The Antarctic krill (*Euphausia superba*) is a key species of the Atlantic sector of the Southern Ocean ecosystem and plays an important role both as feeder of algae, bacteria and microzooplankton and as a prey of vertebrates. It displays a large daily vertical migration that makes a significant amount of biomass available as food for predators near the surface at night and in deeper waters during the day. Despite the great interest on this species, however, the molecular and physiological mechanisms that determine its abundance and distribution are still very poorly understood. The genome sequence of krill is not yet available and therefore the systematic sequencing of cDNA libraries [1] represents a powerful approach to identify large numbers of transcripts that could be used in gene expression and functional genomics studies. To this purpose we produced and pyrosequenced a novel normalized cDNA library characterized by two steps: the “whole transcriptome amplification (WTA)” and “Duplex-specific nuclease (DSN) normalization”. This strategy allowed to optimize the discovery rate of the random sequencing process by equilibrating the final representation of abundant and rare transcripts. To increase the probability of identifying circadian clock genes we constructed a normalized library from krill sampled at different times of the day over a complete 24-hour cycle. Using the 454 Titanium technology we have identified 89,230 high-quality reads which were assembled into 350 overlapping clusters and 10,647 singletons (>200 nt) resulting in a total of 10,987 putative transcripts. Sequences generated by our group and all available *E. superba* sequences from public databases, at present 6,142 ESTs [1, 2] and 777,544 [3] 454 reads (Taxonomy Browser at NCBI, June 2011), have been assembled to create the first krill microarray platform, named Krill 1.2, with a total of 32,217 different probes. We produced 8x60K microarrays (Agilent Technologies) which allowed the analysis of eight different samples on single slides. Using krill 1.2 platform we defined gene expression signatures of specimens collected in the Ross Sea at five different time of the day, during the Antarctic summer, over a complete 24-hour cycle in order to characterize the krill circadian transcriptome [4]. Our work gives a first insight into the molecular mechanisms that allow krill's clock to interpret environmental signals and modulate physiology and behavior accordingly.

REFERENCES:

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