Complex Diel Cycles of Gene Expression in Coral-Algal Symbiosis

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Reef-building corals exhibit complex rhythmic biological behaviors (*1*). Understanding circadian regulation in these organisms is complicated by their photosynthetic endosymbionts (*Symbiodinium* spp.). Photosynthesis by the symbionts results in coral tissue being hyperoxic by day but near hypoxic by night when respiration dominates (*2*). To better understand how corals tune their circadian machinery to respond to external and internal cues, we performed microarray analysis of coral genes (*3*) with *Acropora millepora*. Three coral colonies were sampled at 4-hour intervals over 2 days under ambient light-dark (LD) cycles and under constant darkness (DD) (fig. S1). K-means clustering of the microarray data after Fourier analysis identified several co-regulated (synexpression) clusters consisting of functionally related genes with common temporal expression patterns (figs. S1 to S3 and table S1) (*4*).

A synexpression group of genes that are strictly light-coupled and include coral cryptochromes had an expression peak at midday (zeitgeber time zt6), which corresponds to the period of highest illumination (Fig. 1A) (*1*). The α-type *carbonic anhydrase* (α-CA) in this synexpression cluster potentially underlies the phenomenon of light-enhanced coral calcification (*5*). Other genes in this cluster have known or possible antioxidant functions, including *ferritin*-H, the heme-binding protein 2 (HeBP2), and catalase (fig. S4) (*6*–*8*). These data for corals suggest that the link between the cellular redox state and the circadian clock may be an ancestral trait.

A second group of *A. millepora* genes peaked in expression at midnight (zt18) under normal LD cycles (Fig. 1B) but did not cycle under DD. These genes encode several glycolytic enzymes and other enzymes required for biosynthetic pathways (*4*).

Glycolytic enzymes are regulated by the hypoxia-inducible factor (HIF) 1α transcription factor (*9*), an ortholog of which is present in *A. millepora*. In the coral, HIF expression has a peak at zt14 in LD (fig. S5). The tight temporal clustering of the glycolytic gene group implies regulation by symbiont physiology, which is linked to external light levels.

Molecular chaperones formed another synexpression group that cycles not only under LD (peaking at zt10; 16:00) but also (with the sole exception of *hsp40*) in constant darkness (Fig. 1, C and D). Although chaperones have been linked to the circadian clock (*10*), there are no precedents for extensive coordinated chaperone expression, which probably reflects the diel pattern of stress experienced by symbiotic corals.

Our data may indicate that the HIF system in corals mediates diel cycles in central metabolism, whereas many genes involved in stress responses and protection are coupled to the circadian clock. Our data highlight the physiological complexity of regulatory mechanisms in the coral-algal symbiosis; diel cycles appear to be governed by the circadian clock, with the HIF system most likely operating in parallel.

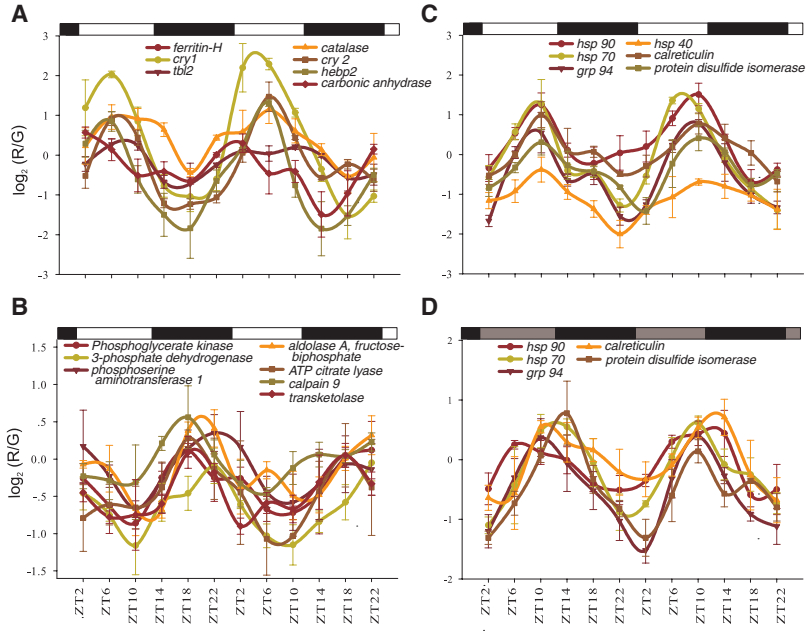

Fig. 1. Temporal gene expression data. RNA samples collected from the symbiotic coral *A. millepora* ($N = 3$) and hybridized to microarray during LD cycles or DD are shown as log ratios of test sample (R)/reference (G) and are plotted according to zeitgeber time; zt2 to zt22 is equivalent to 08:00 to 04:00. Error bars represent the standard error of the mean. (**A**) α–carbonic anhydrase and several antioxidant genes show classical light-dependent circadian regulation, peaking in expression with cryptochromes during the period of highest illumination (zt6 = 12:00). (**B**) Metabolic genes (including three glycolytic enzymes) that peak in expression in the dark (zt18 to zt22 equals 24:00 to 04:00) and may be induced by symbiont-driven hypoxia. (**C** and **D**) A large cluster of chaperones peak in expression at zt10 = 16:00 under both normal (C) LD and (D) DD cycles.

References and Notes

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11. We thank Y. Gothilf for critical discussions and N. Simon-Blecher for assistance in collecting coral samples. This work was supported by the Australian Research Council (O.H.-G. and D.J.M.) and Israel Science Foundation grant no. 699/06. Microarray data are Minimum Information About a Microarray Experiment (MIAME)–compliant and deposited under accession number GSE21658 (National Center for Biotechnology Information GEO). HIF ortholog present in *A. millepora* (GenBank identification EZ037157).

Supporting Online Material

www.sciencemag.org/cgi/content/full/331/6014/175/DC1
Materials and Methods
SOM Text
Figs. S1 to S5
Table S1
References

12 August 2010; accepted 8 December 2010
10.1126/science.1196419

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