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SHORT COMMUNICATION

Evidence for a role of viruses in the thermal sensitivity of coral photosymbionts

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***Symbiodinium*, the dinoflagellate photosymbiont of corals, is posited to become more susceptible to viral infections when heat-stressed. To investigate this hypothesis, we mined transcriptome data of a thermosensitive and a thermotolerant type C1 *Symbiodinium* population at ambient (27 °C) and elevated (32°C) temperatures. We uncovered hundreds of transcripts from nucleocytoplasmic large double-stranded DNA viruses (NCLDVs) and the genome of a novel positive-sense single-stranded RNA virus (+ssRNAV). In the transcriptome of the thermosensitive population only, +ssRNAV transcripts had remarkable expression levels in the top 0.03% of all transcripts at 27 °C, but at 32 °C, expression levels of +ssRNAV transcripts decreased, while expression levels of anti-viral transcripts increased. In both transcriptomes, expression of NCLDV transcripts increased at 32 °C, but thermal induction of NCLDV transcripts involved in DNA manipulation was restricted to the thermosensitive population. Our findings reveal that viruses infecting *Symbiodinium* are affected by heat stress and may contribute to *Symbiodinium* thermal sensitivity.**
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Tropical reef-building corals form a multipartite symbiosis with the photosynthetic dinoflagellate *Symbiodinium* and a diverse microbial community that includes bacteria, archaea, fungi, protists and viruses; collectively termed the coral holobiont (Rohwer *et al.*, 2002). The *Symbiodinium*–coral symbiosis can be disrupted by heat stress, which results in the loss of *Symbiodinium* cells from coral tissues, i.e., coral bleaching (Hoegh-Guldberg, 1999). Heat stress has been alleged to promote lytic viral infections of *Symbiodinium*, as virus-like particles have been found in heat-stressed *Symbiodinium* from the temperate sea anemone *Anemonia viridis* (Wilson *et al.*, 2001) and from the corals *Pavona danai*, *Acropora formosa* and *Stylophora pistillata* (Wilson *et al.*, 2005; Davy *et al.*, 2006). Additionally, following heat shock at 31 °C, the expression of a protein with homology to a eukaryotic viral protein increased >100-fold in a *Symbiodinium*-enriched fraction of *Stylophora pistillata* tissue (Weston *et al.*, 2012).

Using transcriptome data generated by Levin *et al.* (2016) for two *Symbiodinium* type C1 populations cultured at 27 °C and 32 °C ($n=4$), we explore the effect of heat stress on viruses associated with *Symbiodinium* and *Symbiodinium* anti-viral responses. The *Symbiodinium* populations were originally isolated from the coral *Acropora tenuis* at South Molle Island (SM) and Magnetic Island (MI) (Great Barrier Reef, Australia). The thermosensitive SM population was found to suffer physiological damage in culture and to bleach *in hospite* at 32 °C, whereas the thermotolerant MI population was unaffected (Howells *et al.*, 2012; Levin *et al.*, 2016).

As many viral RNAs are polyadenylated (Wilson *et al.*, 2000; Priet *et al.*, 2015), they were retained in the poly(A)+ purified *Symbiodinium* RNA samples used for RNA-Seq (Levin *et al.*, 2016). Viral transcripts in each *de novo* transcriptome were identified through a robust BLASTx bit score approach adapted from Boschetti *et al.* (2012), which calculates the difference between the highest viral and the highest non-viral bit score to determine if a transcript is from a virus

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or the host, followed by GC content, trinucleotide frequency and codon usage analyses (Supplementary Materials and Methods, Supplementary Figure 1 and Supplementary Datasets 1 and 2). *Symbiodinium* anti-viral transcripts were identified by searching the transcriptomes for transcripts encoding anti-viral gene types found in corals and for transcripts with anti-viral Gene Ontology (Supplementary Materials and Methods).

The thermosensitive SM and thermotolerant MI transcriptomes contain 306 and 238 viral transcripts (Supplementary Discussion), as well as 62 and 65 anti-viral transcripts, respectively (NCBI GEO accession: GSE77911). In both transcriptomes, viral transcripts show homology to genes from NCLDVs *Mimiviridae* and *Phycodnaviridae* and the major capsid protein (MCP) gene from the dinoflagellate-specific +ssRNAV *Alvernaviridae* (*Dinornavirus*), which is in agreement with previous sequencing and transmission electron microscopy findings (Wilson *et al.*, 2005; Correa *et al.*, 2013, 2016). The +ssRNAV transcripts in the thermosensitive SM transcriptome share 94% nucleotide (nt) identity (TR74740|c13_g1_i1, 5202 nt; TR74740|c13_g1_i2, 2154 nt). The +ssRNAV transcript in the thermotolerant MI transcriptome is much shorter (TR97578|c0_g1_i1, 475 nt) but shares 100% nt identity with TR74740|c13_g1_i1. Successful PCR amplification of the MCP genes from complementary DNA reverse-transcribed from RNA—but not from genomic DNA—of both *Symbiodinium* populations supports that they are from +ssRNAVs (Supplementary Materials and Methods and Supplementary Figure 2). However, phylogenetic analysis of the translated MCP gene sequences revealed that they are highly divergent from the *Dinornavirus* MCP gene and previously identified partial-length *Dinornavirus*-like MCP genes (Supplementary Discussion, Supplementary Table 1 and Supplementary Figure 3).

Both +ssRNAV transcripts in the thermosensitive SM transcriptome contain a putative viral internal ribosomal entry site (Supplementary Figure 4), which is related to the internal ribosomal entry site of the +ssRNA cricket paralysis virus, directly upstream from the MCP gene. The longer +ssRNAV transcript, TR74740|c13_g1_i1, also encodes an unannotated open reading frame (ORF) determined to be a +ssRNAV RNA replicase (RNA-dependent RNA-polymerase) polyprotein based on protein structure modeling (Supplementary Figure 5). The RNA replicase gene precedes the internal ribosomal entry site and MCP gene, giving the full transcript a markedly similar arrangement to the *Dinornavirus* and cricket paralysis virus complete genomes (Wilson *et al.*, 2000; Nagasaki *et al.*, 2005). Thus, we conclude TR74740|c13_g1_i1 to be the RNA genome of a novel +ssRNAV, making this the first discovered genome of any virus infecting *Symbiodinium* (Figure 1a). Surprisingly, the conserved dinoflagellate spliced leader, which is present on >95% of *Symbiodinium* mRNAs (Zhang *et al.*, 2013), is at the 5′ end of TR74740|c13_g1_i1. Although, if the +ssRNAV is dinoflagellate-specific like *Dinornavirus*, incorporation of the dinoflagellate spliced

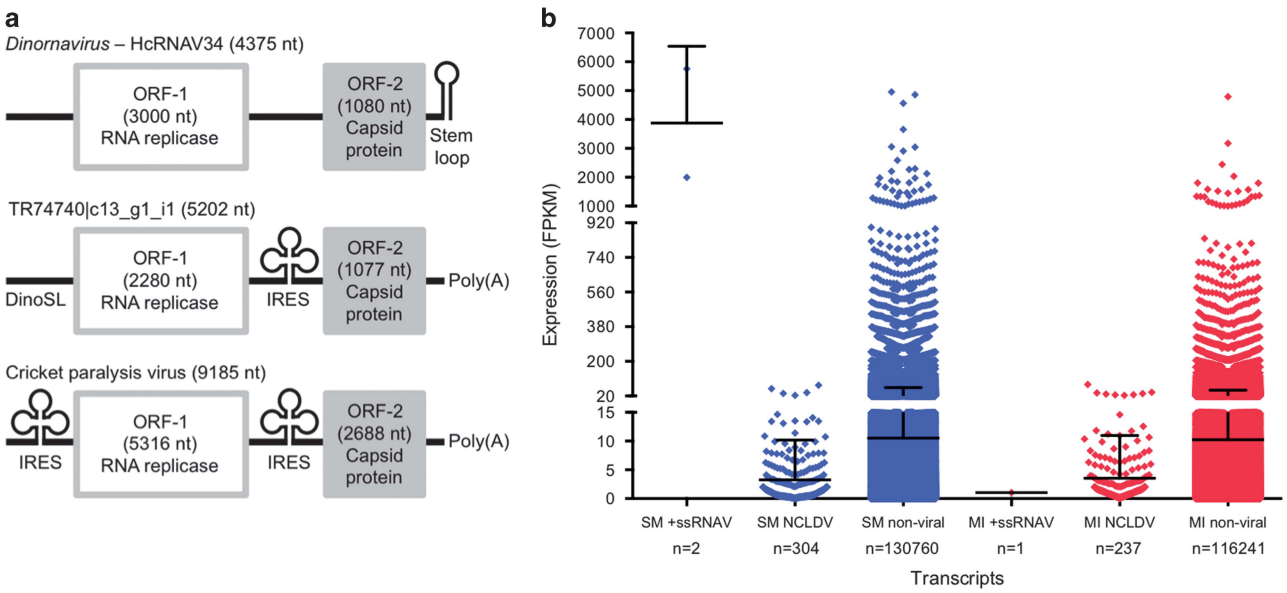

Figure 1 Genome of the novel +ssRNAV and its expression in *Symbiodinium* transcriptomes. (**a**) Genome model of the +ssRNAV infecting *Symbiodinium* (TR74740|c13_g1_i1, GenBank accession: KX538960) and its similarities to the RNA genomes of the dinoflagellate-specific *Dinornavirus* (*Heterocapsa circularisquama* RNA virus strain 34, NCBI accession: AB218608) and cricket paralysis virus (NCBI accession: AF218039). (**b**) TMM-normalized FPKM at 27 °C (averaged across replicates for each transcript on day −1, $n=8$) for the +ssRNAV transcripts (TR74740|c13_g1_i1, 5757 FPKM; TR74740|c13_g1_i2, 1996 FPKM; TR97578|c0_g1_i1, 1 FPKM), NCLDV transcripts and non-viral transcripts in the thermosensitive SM and thermotolerant MI transcriptomes. Black lines mark the mean FPKM +s.d. for each subset of transcripts.

leader in the viral RNA genome is likely a case of molecular mimicry, a well-documented viral strategy to evade host immune responses that detect foreign nucleic acids (Elde and Malik, 2009), or for efficient cap-dependent translation by host polysomes (Zeiner *et al.*, 2003).

Viral transcripts had similar expression levels to many non-viral transcripts at 27 °C, with the exception of the two +ssRNAV transcripts in the thermosensitive SM transcriptome (Figure 1b). Both +ssRNAV transcripts in the thermosensitive SM transcriptome maintained average expression levels >1300 fragments per kb of transcript per million mapped reads (FPKM) on all sampling time points at 27 °C, whereas the +ssRNAV transcript in the thermotolerant MI transcriptome had an average expression level <2 FPKM on all sampling time points. The vastly dissimilar expression levels of +ssRNAV transcripts between the transcriptomes suggest that the thermosensitive SM *Symbiodinium* population was experiencing a severe viral infection.

Differential expression analysis (Supplementary Materials and Methods) confirmed that no viral or anti-viral transcripts were differentially expressed between experimental groups of either population on

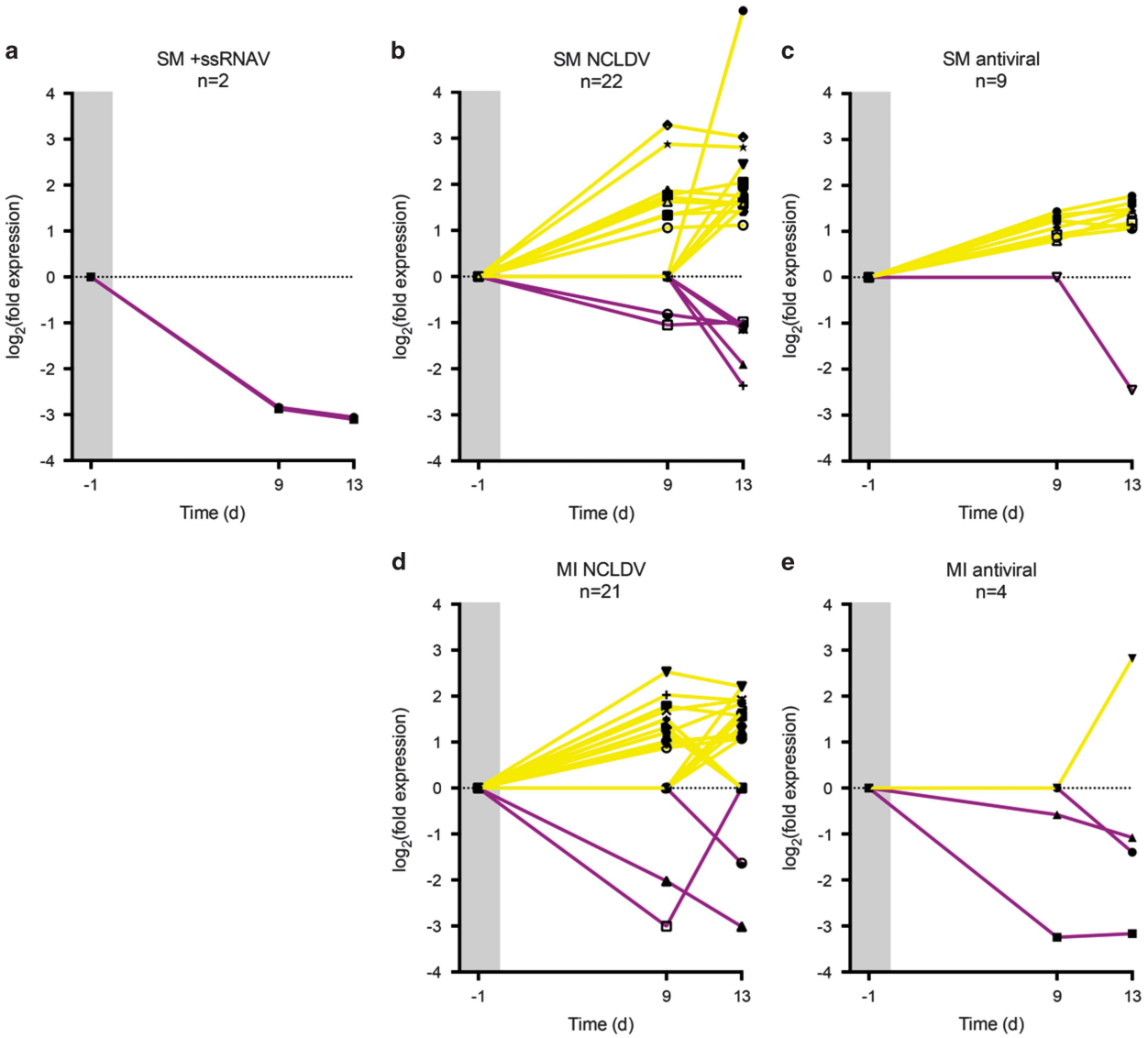

Figure 2 Viral infections and anti-viral responses of *Symbiodinium* under heat stress. Differential expression of (**a**) thermosensitive SM transcriptome +ssRNAV transcripts, (**b**) thermosensitive SM transcriptome NCLDV transcripts, (**c**) thermosensitive SM transcriptome anti-viral transcripts, (**d**) thermotolerant MI transcriptome NCLDV transcripts and (**e**) thermotolerant MI transcriptome anti-viral transcripts at 32 °C. Only transcripts with ⩾2-fold expression between 27 °C and 32 °C treatments on at least one sampling time point were analyzed. Transcripts were considered to have no differential expression on a sampling time point where the false discovery rate (FDR) was >0.001. Upregulated transcripts at 32 °C are shown in yellow. Downregulated transcripts at 32 °C are shown in purple. The gray regions represent the preheating sampling time point on day −1 when all replicates (n=8) were still at 27 °C. On day 0, replicates were ramped to 32 °C (n=4) or maintained at 27 °C (n=4) for the duration of the study (Supplementary Materials and Methods). Differential expression and transcript annotation results are detailed in Supplementary Tables 2–9.

day −1 (preheating; all samples acclimated at 27 °C) (Figures 2a–e). In both transcriptomes, upregulation of NCLDV transcripts was induced at 32 °C and increased from day 9 to day 13 (Figures 2b and d, and Supplementary Tables 2–9). However, the upregulated NCLDV transcripts in the thermosensitive SM transcriptome encoded for a greater diversity of genes (F-box and FNIP repeat-containing proteins, resolvase, transposase, ankyrin repeat protein) compared to those in the thermotolerant MI transcriptome (only F-box and FNIP repeat-containing proteins). Viral F-box and FNIP repeat-containing proteins and ankyrin repeat proteins have possible roles in degrading proteins through protein–protein interactions, countering host defences and exploiting the host's ubiquitin-proteasome system to create an appropriate cellular environment for viral replication (Suhre, 2005; Sonnberg *et al*., 2008; Fischer *et al*., 2010), whereas resolvases and transposases directly participate in DNA manipulation (Iyer *et al*., 2001; Schroeder *et al*., 2009).

In the thermosensitive SM transcriptome, heat stress resulted in downregulation of the highly expressed +ssRNAV transcripts and upregulation of anti-viral transcripts (Figures 2a and c and Supplementary Tables 2–3 and 6–7). Conversely, the thermotolerant MI transcriptome showed no differential expression of its lowly expressed +ssRNAV transcript and downregulation of anti-viral transcripts at 32 °C (Figure 2e, Supplementary Tables 8–9). *Symbiodinium* anti-viral responses may therefore become activated from increased DNA manipulation by NCLDVs or initial upregulation of highly expressed +ssRNAV transcripts at 32 °C that was mitigated by the sampling time points on days 9 and 13.

Our study exemplifies how RNA-Seq data can be used to gain valuable insight into resident viruses. Our results indicate that only the thermosensitive SM *Symbiodinium* population experienced an extreme +ssRNAV infection and thermally induced NCLDV DNA manipulation. Thus, viral infections may factor into *Symbiodinium* thermal sensitivity, and consequently, coral bleaching.

Conflict of Interest

The authors declare no conflict of interest.

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