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Supplemental Information

The compound packaged in virions is the key to trigger host glycolysis machinery for virus life cycle in the cytoplasm

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Transparent Methods

Shrimp culture and WSSV infection

Marsupenaeus japonicus shrimp with an average weight of 15 g, collected from a shrimp aquaculture farm at Hangzhou of Zhejiang province of China, were cultured in groups of 20 individuals in air-pumped circulating seawater at 25 ℃. Three shrimp were randomly selected for PCR detection of WSSV with WSSV-specific primers (5'-TTGGTTTCATGCCCGAGATT-3' and 5'-CCTTGGTCAGCCCCTTGA-3') to ensure that the shrimp used were WSSV free before experiments. The virus-free shrimp were infected with WSSV (10^5 copies/ml) by injection (100 μl WSSV inoculum/shrimp) into the lateral area of the fourth abdominal segment. At different times after infection, the WSSV-infected shrimp were collected for later use.

Purification of intact WSSV virions

The tissues of WSSV-infected shrimp excluding hepatopancreas were collected in an ice-bathed beaker and then homogenized in 200 ml TNE buffer [50 mM Tris–HCl, 400 mM NaCl, 5 mM EDTA (ethylene diamine tetraacetic acid), pH 8.5] containing protease inhibitor phenylmethanesulfonyl fluoride. After centrifugation at 5,000×g for 10 min at 4 ℃, the supernatant was filtered through a nylon net (400 mesh). The filtrate was centrifuged at 30,000×g for 30 min at 4 ℃. Subsequently the upper loose pellet was rinsed out carefully, and the lower white pellet was resuspended in 50 ml TM buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, pH 8.0). After centrifugation at 5,000×g for 10 min at 4 ℃, the virus particles were sedimented by centrifugation at 30,000×g

for 30 min, followed by resuspension with TM buffer. The purity of the virions was evaluated using negative-staining transmission electron microscopy (TEM).

Metabolite analysis

The WSSV-infected shrimp hemocytes or the purified WSSV virions (10^{10} copies) were mixed with 99% methanol containing 0.05 mM 2-chloro-L-phenylalanine as an internal standard. The mixture was homogenized for 2 min and centrifuged at 12000×g at 4 ℃ for 10 min. Subsequently the supernatant (the extracted metabolites) was analyzed by liquid chromatography-coupled mass spectrometry (LC-MS) for metabolic profiling using Agilent 1290 Infinity II UHPLC system and Agilent 6545 UHD and Accurate-Mass Q-TOF/MS (Agilent Technologies, Santa Clara, CA, USA). The ESI-MS (electrospray ionization-mass spectrometry) was acquired in the positive ion mode. The product ions of m/z ranging from 50 to 1500 were collected. The heated capillary temperature was maintained at 325℃. The drying gas and nebulizer nitrogen gas flow rates were 10 L/min and 20 psi (pounds per square inch), respectively. The molecular feature extractor (MFE) algorithm within Mass Hunter Qualitative analysis software (Agilent Technologies, USA) was used to extract chemically qualified molecular features. For metabolite identification empirical formula generation, the molecular formula generator (MFG) algorithm was used to generate empirical formula and search against METLIN metabolite compound database (https://metlin.scripps.edu/) (Tautenhahn et al., 2012). Data processing was performed on each individual sample. The metabolite existed in all three samples of

each group was retained. According to the results of paired t-test analysis, the candidate differential metabolites were chosen according to the criteria (p<0.01 and fold change>3). To confirm the identified metabolites, pure standards of highest available purity (Sigma, USA) were prepared in 50% methanol. Then, the standard metabolite solution was analyzed with LC-MS. Matched retention time and mass spectral patterns of the standards and samples were compared.

Effects of compounds on WSSV infection in shrimp

WSSV (10^5 copies/ml) and the compound purchased from Sigma (USA) at different concentrations (1, 10 or 100 μM) were co-injected into virus-free shrimp at a volume of 100 μl per shrimp. The compounds were dissolved in dimethyl sulfoxide (DMSO). WSSV alone (10^5 copies/ml) and physiological saline (0.85% NaCl) were also injected into shrimp. At different times post-infection (0, 24, 36 and 48 h), three shrimp were randomly collected for each treatment and subjected to the detection of WSSV copies. The shrimp mortality was monitored every day. All assays were biologically repeated three times.

Detection of WSSV copies by quantitative real-time PCR

WSSV copies in shrimp were determined by quantitative real-time PCR as described before (Cui et al., 2015). DNA extracted from shrimp hemocytes was subjected to quantitative real-time PCR with WSSV-specific primers (5'-CCACCAATTCTACTCATGTACCAAA-3';
5'-TCCTTGCAATGGGCAAAATC-3') and probe

(5’-FAM-TGCTGCCGTCTCCAA-Eclipse-3’). A linearized plasmid containing a 1400-bp DNA fragment from the WSSV genome was quantified and serially diluted as an internal standard.

Transcriptome sequencing

A total of 1.5 μg RNA per sample was used for RNA sequencing. Sequencing libraries were generated using NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (New England Biolabs, USA) following manufacturer’s recommendations and index codes were added to attribute sequences to each sample. Briefly, mRNA was purified from the total RNA using poly-T oligo-attached magnetic beads. Fragmentation was carried out using divalent cations under elevated temperature in NEB next first strand synthesis reaction buffer (5×). The first-stranded cDNA was synthesized with random hexamer primer and M-MuLV reverse transcriptase (RNase H). The second-stranded cDNA synthesis was subsequently performed using DNA polymerase I and RNase H. Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of 3’ ends of DNA fragments, NEB next adaptor with hairpin loop structure were ligated to prepare for hybridization. In order to select cDNA fragments of preferentially 250~300 bp in length, the library fragments were purified with AMPure XP system (Beckman Coulter, Beverly, USA). Then 3 μl USER enzymes (New England Biolabs) were used with size-selected adaptor-ligated cDNA at 37 °C for 15 min, followed by 5 min at 95 °C before PCR. PCR was performed with phusion high-fidelity DNA polymerase, universal PCR

primers and index primer. PCR products were purified (AMPure XP system) and library quality was assessed on the Agilent Bioanalyzer 2100 system.

Kyoto encyclopedia of genes and genomes (KEGG) analysis

The coding sequences of transcripts were extracted and used as queries to search the protein sequences collected in the GO (gene ontology) database with the blast E-value of less than 1×10^{-5}. The best hit GO identities were assigned to the transcripts. The p-values were corrected for false discovery rate. Deduced genes with homologues in other organisms were used to map to conserved biological pathways.

Identification of proteins bound to palmitic amide

To obtain the proteins interacted with palmitic amide, palmitic amide was labeled with biotin according to the manufacturer's instructions (Thermo Scientific, USA). Then the biotin-labeled palmitic amide was coupled to Dynabeads MyOne Streptavidin T1 (GE Healthcare, USA). Shrimp hemocytes were lysed with cell lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, pH 7.5) on ice for 2 h. After centrifugation at 15,000×g for 20 min, the supernatant was incubated with 1 mL of palmitic amide-coupled beads or negative control (Dynabeads alone) overnight at 4 °C with gentle rotation. The beads were washed five times with PBS to remove non-specific proteins. The mixture containing bound proteins was analyzed with SDS-PAGE. Proteins were visualized using Coomassie blue staining. The visible interested protein bands were excised from SDS-PAGE gel and subjected to protein identification by mass spectrometry (Bruker Daltonics, USA).

RNAi (RNA interference) assay

Small interfering RNA (siRNA) was designed and synthesized in vitro using a T7 kit according to the manufacturer's manual (TaKaRa, Japan). The siRNAs (TPI-siRNA, 5'-UGCUGACGAUCUUCUUGC-3'; TPI-siRNA-scrambled, 5'-CCAGCCU ACAAGAUUCCCAA-3'; HIF-1-siRNA, 5'-UACUAUCCUUCCCUGUACG-3'; HIF-1-siRNA-scrambled, 5'-AUGUACUGAGCUAAUUGCG-3') were dissolved in siRNA buffer (50 mM Tris-HCl, 100 mM NaCl, pH 7.5) and quantified by spectrophotometry. RNAi assay was conducted in shrimp by the injection of a siRNA (30 μg/shrimp) into the lateral area of the fourth abdominal segment using a syringe with a 29-gauge needle. At different times after injection, the shrimp hemocytes were collected for later use. The assays were biologically repeated for three times.

Northern blotting

Total RNAs were extracted using a mirVana miRNA isolation kit according to the manufacturer's instructions (Ambion, USA). Subsequently the total RNAs were electrophoresed in a denaturing 15% polyacrylamide gel containing 8 M urea and transferred to a Hybond-N membrane (Amersham Biosciences, Buckinghamshire, UK). After cross-linking with ultraviolet, the membrane was prehybridized in DIG (digoxigenin) Easy Hyb granule buffer (Roche, Switzerland) for 0.5 h, followed by hybridization with DIG-labeled TPI probe (5'-GGTCAGGCTCCCCGAAGAC-3') or HIF-1 probe (5'-CGAGGAGCGAAGATCTTGGATGTG-3') for 20 h at 45 ℃. Shrimp β-actin probe (5'-CTCGCTCGGCGGTGGTCGTGA-3') was included as a

control. Signal detection was performed following the instructions for a DIG High Prime DNA labeling and detection starter kit II (Roche, Switzerland).

Isothermal titration calorimetry (ITC)

The titration experiments were conducted on a VP-ITC platform (MicroCalTM, Inc., Northampton, MA, USA) at 25 °C to characterize the protein-compound interaction. The protein (at a final concentration of 100 μM) in the cell of the VP-ITC platform was added with a compound (1 mM) at 10 μL/120 s. All experiments were conducted at least in triplicate. The data were analyzed using Origin 7 and fitted to a "One-Sites" model (MicroCalTM Inc.). The thermodynamic association constant (Ka) and enthalpy change (ΔH) were calculated directly. The dissociation constant (Kd) was calculated according to the equation Kd = 1/Ka. The Gibbs free energy change (ΔG) was calculated using the equation $\Delta G = -RT\ln Ka$, where R was the molar gas constant and T was the absolute temperature at which the experiment was conducted. The entropy change (ΔS) of the interaction was calculated according to the equation $T\Delta S=\Delta H-\Delta G$.

Examination of glycolysis

To examine the shrimp glycolysis, shrimp hemocytes were collected, followed by the quantification of glucose and lactate contents. The hemocytes were centrifuged at 300×g for 10 min at 4 °C. Subsequently the concentration of glucose or lactate of the supernatant was determined using glucose assay kit (Beijing Solarbio Science & Technology Co., Ltd., China) or lactate assay kit (Solarbio, China). Briefly 20 μL of

sample was mixed with 200 μL of glucose or lactate working reagent in a single well of a 96-well plate. After incubation at 37 °C for 15 min for glucose or at 25 °C for 5 min for lactate, the absorbance of the sample was measured at 505 nm (glucose) or 570 nm (lactate). To quantify the content of glucose or lactate, a standard curve was generated. Subsequently the absolute content of glucose or lactate was determined.

Western blot

Proteins were separated using 12% SDS-PAGE and then transferred to a polyvinylidene fluoride (PVDF) membrane (Amersham Biosciences, UK). The membrane was blocked with TBST (Tris-buffered saline with Tween 20) containing 5% skimmed milk. Subsequently the membrane was incubated overnight with a primary antibody, followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibody (Sigma-Aldrich, USA) for 2 h at room temperature. The primary antibody was prepared in our laboratory. The proteins were detected using Western Lightning Plus-ECL Oxidizing Reagent Plus (PerkinElmer, USA).

Detection of TPI enzymatic activity

Cytoplasmic proteins were prepared using the NE-PER protein extraction kit (Pierce, Rockford, IL, USA). The protein concentration was measured with the Bradford protein assay (Bio-Rad Laboratories, Hercules, CA, USA). The enzymatic activity of TPI was determined as described previously (Olivaresillana et al., 2017), based on the conversion of glyceraldehyde 3-phosphate into dihydroxyacetone

phosphate 2. The cytoplasmic proteins (5 ng/mL) were mixed with the reaction buffer [100 mM triethanolamine, 10 mM ethylene diamine tetraacetic acid (EDTA), 0.2 mM nicotinamide adenine dinucleotide (NADH), 1 mM glyceraldehyde 3-phosphate (GAP) and 20 μg of α-glycerol phosphate dehydrogenase (α-GPDH)] at a final volume of 1 mL. After incubation at 25 °C for 5 min, the absorbance of the sample was measured at 340 nm with a plate reader. To quantify TPI enzymatic activity, a standard curve was generated. The TPI activity unit was determined as the micromoles of NADH formed per min per mg of protein at 25 °C.

Examination of tricarboxylic acid cycle (TCA)

To examine Sanchez the host's TCA, the contents of acetyl CoA and mitochondrial citric acid of hemocytes were measured using the acetyl CoA assay kit (Beijing Solarbio Science & Technology Co., Ltd., China) and the mitochondrial citric acid assay kit (Solarbio, China), respectively. Briefly 20 μL of sample was mixed with 200 μL of working reagent (Solarbio, China) in a single well of a 96-well plate. After incubation at 37 °C for 30 min, the absorbance of the sample was measured at 330 nm with a plate reader.

Statistical analysis

The numerical data from three independent experiments were analyzed by one-way analysis of variance (ANOVA). The differences between treatments were analyzed by Student's t-test.

Supplemental References

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