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LETTER

3'-UTR sequence of *Macrobrachium rosenbergii* extra small virus (XSV) is important for viral RNA packaging

Dear Editor,

The packaging of viral genomic RNA into virus particles is a critical step for virus maturation. This step includes the recognition and interaction between the nucleocapsid protein and viral RNA. The necessity of viral RNA packaging signals has been described for many RNA viruses (Cologna R, et al., 2000; Narayanan K, et al., 2001; Tchatalbachev S, et al., 2001). Occasional packaging of nongenomic viral RNAs and cellular RNAs results in noninfectious virions, and casual packaging of cellular RNA into viral particles would overwhelm the ability of viral genomic RNA to contact limited assembly factors. Therefore, viruses have probably developed a defensive strategy for the specific packaging of genomic RNA into virus particles. The packaging signals of some RNA viruses are located within more than one viral genomic region (Luytjes W, et al., 1989; Pattnaik A K, et al., 1995; Wei N, et al., 1990). For many viruses, it is unknown whether a single region or a combination of multiple genomic regions is necessary for viral RNA packaging.

The giant freshwater prawn *Macrobrachium rosenbergii* is one of the most economically important farmed palaemonids in the world. A viral disease called white tail disease (WTD) has affected *M. rosenbergii* in Thailand, Guadeloupe, the Antilles, China, and India since the early 1990s (Qian D, et al., 2003; Yoganandhan K, et al., 2006), and these outbreaks have resulted in significant economic losses. Two viral particles, *M. rosenbergii* nodavirus (*Mr*NV) and its satellite virus extra small virus (XSV), were isolated and identified from the diseased prawns (Qian D, et al., 2003). Experimental infections demonstrated that *Mr*NV plays a key role in WTD and that XSV is dependent on *Mr*NV during replication (Bonami J R, et al., 2011; Zhang H, et al., 2006). The XSV is 15 nm in diameter, icosahedral, and nonenveloped. The viral particle consists of two structural proteins (CP17 and CP16) and encapsulates a linear single-stranded RNA genome of 0.9 kb (Shi Z, et al., 2004). CP17 and CP16 are independently translated initiating from different start codons in the same open reading frame (ORF) (Wang J, et al., 2008) and are at almost equal molarity in the viral particles. We previously constructed plasmids containing CP17, CP16, or truncated CP17 and expressed them in *Escherichia coli*. All constructs, except the one with the deletion of 10 amino acids at the C-terminal, could assemble into virus-like particles (VLPs) (Wang J, et al., 2008), suggesting that the C-terminal is important for VLP formation.

In this study, the plasmid pET28a-CP17 expressing XSV CP17 protein was prepared as described previously (Wang J, et al., 2008). The full-length and truncated XSV genomic cDNA (with deletion at the 5'-UTR or 3'-UTR) were amplified from cDNA fragments of the XSV genome previously prepared in our laboratory (GenBank accession no: DQ174318) (Zhang H, et al., 2006), using primers listed in supplementary Table 1, and cloned into a pGEM-T easy vector. The resulting constructs were named pGEM-XSV_full, pGEM-XSV_del5', and pGEM-XSV_del3', respectively.

The pET28a-CP17 plasmid was transformed in *E. coli* alone or together with pGEM-XSV_full, pGEM-XSV_del5', or pGEM- XSV_del3'. Cells were harvested after 4 h incubation with 0.5 mmol/L IPTG at 37 °C and precipitated by centrifugation (5,000×g, at 4 °C for 10 min). The harvested bacteria were resuspended in DNase I reaction buffer (40 mmol/L Tris-Cl [pH 7.5], 8 mmol/L $MgCl_2$, 5 mmol/L DTT) with 1% Triton and 1 mg/mL lysozyme. After five freeze-thaw cycles and digestion by 100 μg/mL DNase I and 100 μg/mL RNaseA at 37 °C for 1 h, cell debris was removed by centrifugation (13,000×g, at 4 °C, for 30 min). The supernatant was ultracentrifuged on a sucrose cushion at 50,000 rpm for 2 h using a Ty70 rotor (Beckman Coulter, Brea, CA, USA). Fractions were collected, washed, resuspended in DNase I reaction buffer, and treated again with DNase I and RNaseA. The purified VLPs were observed under electron microscopy (EM) as described previously (Wang J, et al., 2008).

Purified VLPs were run on a 12% SDS–PAGE gel and transferred to a PVDF membrane. The membrane was blocked for 2 h with 3% skim milk powder in Tris-buffered saline (TBS), washed three times with TBS with 0.1% Tween-20 (TBST) and blotted with CP17 polyclonal antibody (1:1000) produced in our laboratory (Wang J, et al., 2008). Total RNA was extracted from purified VLPs using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and used for electrophoresis or RT-PCR using the

primers listed in supplementary Table 1.

The CP17 proteins were expressed in a soluble form (Figure 1B, upper panel) and assembled into VLPs (Figure 1A) in *E. coli* regardless of transformation of the pET28a-CP17 plasmid with full-length or truncated XSV genomic cDNA plasmids (data not shown). The western blot results showed that all the recombinant proteins presented as a single band at 17 kDa, which corresponded to CP17 (Figure 1B, bottom panel). All VLPs displayed a size of 14–15 nm in diameter with a similar morphological structure to XSV native particles (Shi Z, et al., 2004).

The electrophoresis of RNA extracted from VLPs indicated that all VLPs could package RNA of a similar size of 0.9 kb (Figure 1C, upper panel). However, RT-PCR could only detect the XSV genomic RNA in VLPs cotransformed with full-length or 5'-terminus-deleted genomic cDNA plasmid, not the 3'-terminus-deleted cDNA plasmid (Figure 1C, bottom panel). These results strongly suggested that the 3'-terminus of the XSV genome contains the sequence required for genomic RNA packaging.

It is well known that the 5'- and 3'-termini of virus genomes play important roles in RNA genome packaging and viral particle assembly in most viruses (Cologna R, et al., 2000; Pattnaik A K, et al., 1995). In this study, CP17 and cotransformed CP17 with UTR-deficient XSV in *E. coli* cells could assemble into VLPs that displayed a similar structure and size under EM as natural viral particles. Interestingly, we found that nonviral mRNA was also packaged in VLPs when we only transformed the CP17-expressing plasmid and CP17 cotransformed with 5'UTR-deficient XSV. A similar phenomenon has been observed in other viruses (Onafuwa-Nuga A A, et al., 2005; Rulli S J, Jr., et al., 2007; Schopman N C, et al., 2012). For retroviruses, some cellular mRNAs are selectively packaged in viral particles and have diverse functions in virus replication. However, the mechanism of cellular RNA packaging in viral particles remains largely unknown for many viruses. It will be valuable to further investigate the nonviral sequences packaged in viral particles using high-throughput sequencing techniques and assess the interaction mechanism between viral proteins and packaged RNA sequences.

FOOTNOTES

The supplementary Table 1 is linked to the online version of the paper on the website of Virologica Sinica: http://www.virosin.org.

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All the authors declare that they have no competing interest.

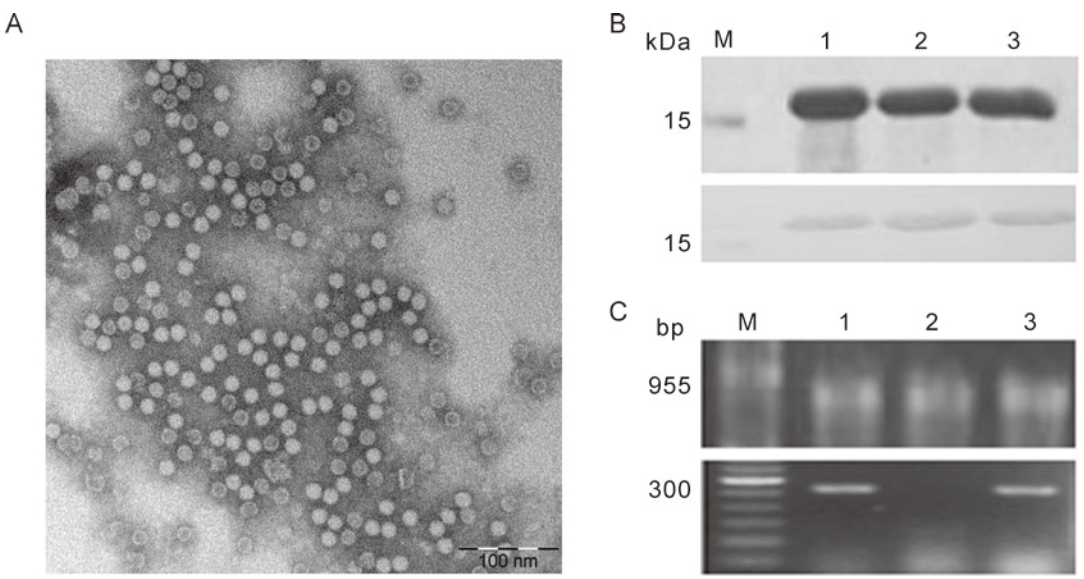

Figure 1. Expression of XSV CP17 protein and detection of viral RNA by RT-PCR. The plasmid containing the XSV *CP17* gene was cotransformed in *E. coli* with a plasmid containing full-length XSV or truncated genomes. Purified VLPs (Figure 1A) were run on a 12% SDS–PAGE gel (Figure 1B, top panel) and detected with polyclonal antibody against CP17 protein (Figure 1B, bottom panel). Packaged viral RNA was detected by electrophoresis (Figure 1C, top panel) and RT-PCR (Figure 1C, bottom panel). Primers targeting the XSV 5'-UTR were used to detect the packaged viral genome with the 3' truncation (Figure 1C, lane 2), and those targeting the XSV 3'-UTR were used to detect the packaged full-length genome and that with the 5' truncation (Figure 1C, lanes 1 and 3). M, molecular marker of protein (Figure 1B) or DNA (Figure 1C).

This article does not contain any studies with human or animal subjects performed by the any of the authors.

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REFERENCES

Bonami J R, Sri Widada J. 2011. J Invertebr Pathol, 106: 131-142.
Cologna R, Hogue B G. 2000. J Virol, 74: 580-583.
Luytjes W, Krystal M, Enami M, et al. 1989. Cell, 59: 1107-1113.
Narayanan K, and Makino S. 2001. J Virol, 75: 9059-9067.
Onafuwa-Nuga A A, King S R, Telesnitsky A. 2005. J Virol, 79: 13528-13537.
Pattnaik A K, Ball L A, LeGrone A, et al. 1995. Virology, 206: 760-764.
Qian D, Shi Z, Zhang S, et al. 2003. J Fish Dis, 26: 521-527.
Rulli S J, Jr., Hibbert C S, Mirro J, et al. 2007. J Virol, 81: 6623-6631.
Schopman N C, van Montfort T, Willemsen M, et al. 2012. Virus Res, 169: 438-447.
Shi Z, Qian D, Zhang J. 2004. Chin J Viro1, 20: 58-61.
Tchatalbachev S, Flick R, Hobom G. 2001. Rna, 7: 979-989.
Wang J, Zhang H, Shi Z. 2008. Virol Sin, 23: 73-77.
Wei N, Heaton L A, Morris T J, et al. 1990. J Mol Biol, 214: 85-95.
Yoganandhan K, Leartvibhas M, Sriwongpuk S, et al. 2006. Dis Aquat Organ, 69: 255-258.
Zhang H, Wang J, Yuan J, et al. 2006. Dis Aquat Organ, 71: 11-17.