Songklanakarin J. Sci. Technol.
33 (2), 121-127, Mar. - Apr. 2011

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Original Article

Cellular targets and pathways of yellow head virus infection in lymphoid organ of *Penaeus monodon* as studied by transmission electron microscopy

Pornsawan Duangsuwan[1], Yotsawan Tinikul[2,3], Boonsirm Withyachumnarnkul[3,4], Charoonroj Chotwiwatthanakun[2,3], and Prasert Sobhon[3,*]

[1] *Department of Anatomy, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.*

[2] *Mahidol University, Nakhon Sawan Campus, Nakhon Sawan 60000, Thailand.*

[3] *Department of Anatomy,*

[4] *Center of Excellence for Shrimp Molecular Biology and Biotechnology, Faculty of Science, Mahidol University, Phaya Thai, Bangkok, 10400 Thailand.*

Received 5 October 2010; Accepted 28 April 2011

Abstract

Negative-stained intact yellow head virus (YHV) was an enveloped bacilliform particle measuring 40-50 x 175-210 nm with spike-like projections measuring 7-9 nm. The space between projections was 4-7 nm. YHV nucleocapsid was rod-shaped, measuring 35-40 x 160-200 nm, and the RNA genome had 40-50 turns in a helical structure. YHV infected both stromal matrix cells and haemocytes in the lymphoid tubule wall. The patterns of localisation of viral particles were similar in both cells. The fully enveloped viral particles were detected at the cell membrane, endosome, rough endoplasmic reticulum, Golgi complex and secretory vesicles, and virions were exocytosed at the cell membrane. In the case of severe infection, unenveloped viral particles could be detected in the cytoplasm, and they might be released by general breakdown and lysis of the highly infected cells.

Keywords: *Penaeus monodon*, lymphoid organ, yellow head virus, electron microscopy, cellular pathway

1. Introduction

Yellow head virus (YHV) is a serious pathogen to penaeid prawn. It was named from the light yellow coloration of cephalothorax, which is caused by the yellow color of underlying hepatopancreas showing through the translucent carapace of moribund prawn (Boonyaratpalin *et al*., 1993; Chantanachookin *et al*., 1993; Flegel, 2006). YHV was first reported in the black tiger prawn, *Penaeus monodon*, which is commonly cultured in Thailand, in 1990 (Boonyaratpalin *et al*., 1993). It causes extensive mortality and massive production losses to the aquaculture industry in Thailand and either South-east Asian countries. YHV can also naturally or experimentally infect other penaeid species (for review see Munro and Owens, 2007).

Yellow head virus is an enveloped, rod-shaped particle (approximately 40x170 nm in size) with prominent surface projections (11 nm) and an internal striated nucleocapsid (Chantanachookin *et al*., 1993; Nadala *et al*., 1997; Wang and Chang, 2000). YHV-infected cells are of both ectoderm and mesoderm origins (Boonyaratpalin *et al*., 1993; Chantana-

* Corresponding author.
Email address: scpso@mahidol.ac.th

chookin *et al*., 1993; Kasornchandra and Boonyaratpalin, 1995). The main organ being infected by this virus is the lymphoid organ (Chantanachookin *et al*., 1993; Kanobdee *et al*., 2002; Soowannayan *et al*., 2002) that forms lymphoid spheroids in chronic infection (Spann *et al.*, 1997; Hasson *et al.*, 1999; Anggraeni and Owens, 2000; Kanobdee *et al*., 2002; Soowannayan *et al*., 2002; Anantasomboon *et al.*, 2008; Duangsuwan *et al.*, 2008; and for review see Rusaini and Owens, 2010). Futhermore, infected lymphoid organ shows extensive abnormalities, including many degenerated cells in the wall of the tubules whose lumen becomes occluded. The affected cells exhibit hypertrophic nuclei, pyknotic nuclei, and cytoplasmic basophilic inclusions (Chantanachookin *et al*., 1993; Flegel, 2006).

Kasornchandra and Booyaratpalin (1995) who studied the replication of YHV in *P. monodon* by transmission electron microscope (TEM), mistakenly claimed that this virus had double-stranded DNA genome. Furthermore, these authors believed that YHV replicated in the nucleus, while viral assembly occurred in cytoplasm. After replication, progeny nucleocapsids were formed and released directly into cytoplasm through the rupture of the nuclear membrane. However, Chantanachookin *et al*. (1993) reported that there was no clear evidence of nuclear membrane fragmentation during the release of viruses into the cytoplasm. Instead, they suggested that nucleic acid cores were expelled from the nucleus as straight filaments, which acquire envelopes during their passage through the endoplasmic reticulum before being fractured into small viruses. Later works showed that YHV was actually a positive-sense, single-stranded RNA virus (Cowley *et al*., 2000; Sittidilokratna *et al*., 2002). Furthermore, positive-stranded RNA viruses were shown to replicate in the cytoplasm of host cells (Schwartz *et al*., 2004), but it is still not clear how complete YHV particles are assembled. Furthermore, targeted cells and the process of viral entry into the cells of the lymphoid organ is still unclear. Hence this study reported on the mode of entry and the assembly of YHV in the infected cells the lymphoid tubules.

2. Materials and Methods

2.1 YHV inoculums

The inoculum for YHV was kindly provided by Charoen Pokphand Shrimp Culture Research Center, Thailand. This viral inoculum was originally obtained from moribund *P. monodon* showing signs of yellow head disease, with light yellow coloration of the dorsal surface of cephalothorax and generally pale body, that were collected from a farm in Chachoengsao Province, Thailand, in July 1998. The inoculum was confirmed to be YHV by RT-PCR using the IQ 2000 detection kit (Farming IntelliGene Technology Corporation, Taipei, Taiwan), which shows the bands at 277 and/or 777 base pairs. It was kept at -20°C until used.

2.2 Experimental YHV infection

Juvenile *P. monodon* (300 animals aged 3 months, approximately 20 g each) were purchased from a Thai commercial farm. They were confirmed to be negative for white spot syndrome virus (WSSV), Taura syndrome virus (TSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), hepatopancreatic parvovirus (HPV), and yellow head virus (YHV) infections by RT-PCR using the IQ 2000 detection kit. Animals were acclimated in aerated aquaria maintained at 28 to 30°C, and fed with commercial feed pellets for 1 week. In total, 290 prawns were intramuscularly injected with 0.1 ml of the YHV inoculums diluted at 1:100 with lobster hemolymph medium (LHM) (Paterson and Stewart, 1974). Ten prawns were injected with only the LHM as a control.

2.3 YHV and nucleocapsid purification

The viruses were purified from hemolymph collected from 200 YHV-infected shrimp at 72 h post infection by ultracentrifugation in a continuous Urografin^{TM} gradient (Schering Pty. Ltd, Midrand, Germany) as described previously (Wongteerasupaya *et al*., 1995; Sittidilokratna *et al*., 2002). A well-defined white band was collected from the gradient at a density corresponding to 25-27% Urografin^{TM}. The YHV envelopes were removed from the viral particles by incubating purified YHV suspension in 1% Triton X-100 in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 7.4) for 30 min at room temperature with gentle shaking. The suspension was centrifuged at 100,000 x g for 1 h at 4°C. The supernatant was then discarded and the pellet (nucleocapsid) was gently resuspended in TE buffer. The purified YHV viruses and nucleocapsids were dropped on carbon-coated formvar film on 400 mesh copper grids, and negatively stained with 4% uranyl acetate in distilled water.The specimens were examined for detailed organization under Tecnai G^2 transmission electron microscope at 100 kV. These were compared with the nucleocapsids and complete viral particles present in the infected cells.

2.4 Specimen preparation for TEM

After injection of the viruses or LHM, ten prawns were sacrificed at every time point from both control and experimental groups at 0, 6, 12, 18, 24 hour and thereafter at 12 hour intervals until all of the shrimp died. Dissected lymphoid organs were fixed in 4% glutaraldehyde and 1% paraformaldehyde in cacodylate buffer, postfixed in 1% OsO_4, dehydrated through ascending concentrations of ethanol and embedded in Araldite-502 resin. Ultrathin sections were cut and stained with uranyl acetate and lead citrate before being observed under a Tecnai G^2 transmission electron microscope at 100 kV.

3. Results

3.1 Ultrastructure of the yellow head viral particle and its nucleocapsid

Negative-stained intact YHV virions were rod-shaped and measured 40-50 nm x 175-210 nm. Viral particle was surrounded by envelope with spike-like projections measuring 7-9 nm. The spacing between the projections was 4-7 nm (Figure 1a). Negative-stained YHV nucleocapsid was a rod-shaped structure measuring 35-40 x 160-200 nm, and had 40-50 turns of RNA with helical appearance (Figure 1b). The combined structure is illustrated in the accompanying diagram (Figure 1c).

3.2 Pathway of yellow head virus in lymphoid cells of *P. monodon*

Haemocytes and stromal cells are the major cell types in the lymphoid tubule wall (Figure 2a). A large number of haemocytes was observed in lymphoid lumen and tubules (Figure 2b) at 6 hours post-infection (h PI). Immature haemocytes were the largest group, accounting for about 40% of total haemocytes. Large granular, small granular and hyaline haemocytes (as classified by Van de Braak *et al*., 1996) comprised about 10%, 30% and 20% of the total haemocytes, respectively (Figure 2b). Haemocytes (Figure 2c) and stromal cells (Figure 2d) were both infected by YHV. The patterns of the localisation of the viral particles were similar in both cells. The fully enveloped YHV particles with approximate size of 40x190 nm were first detected binding to the cell membrane of lymphoid tubule, stromal cells (Figure 3a) and in endosomal-liked vesicles underneath the cell

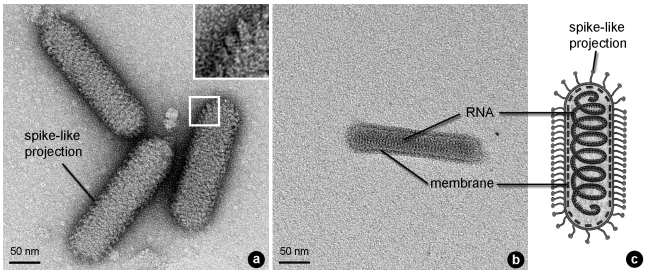

Figure 1. TEM micrographs and a schematic diagram of intact virus and nucleocapsid of the yellow head virus.
a) A high magnification micrograph of the intact YHV viruses, showing enveloped, bacilliform particles with spike-like projections surrounding each virus (shown at high magnification in the inset)
b) High magnification micrograph of YHV nucleocapsid, which has a rod-shaped and a helical structure with 40-50 turns.
c) A diagram illustrating the combined structure of the whole viral particle.

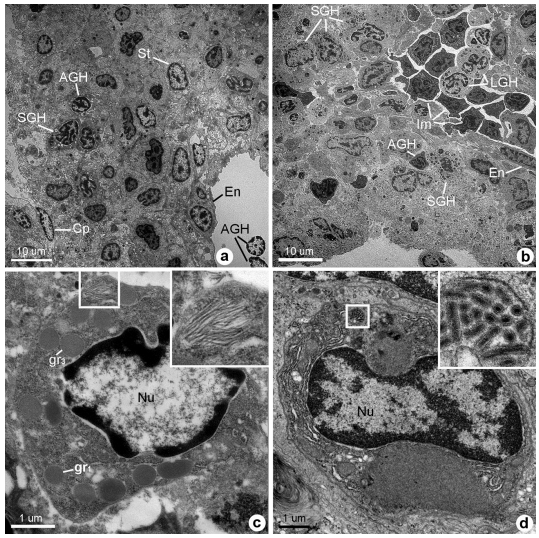

Figure 2. TEM micrographs of control and YHV-infected lymphoid cells and tubules.
a) A low magnification micrograph of a control lymphoid tubule, showing 3 types of fixed cells: endothelial (En), stromal (St), and capsular (Cp) cells, and relatively few agranular (AGH) and small granular (SGH) haemocytes in the wall of the lymphoid tubule. Few agranular (AGH) haemocyte were also observed in the lumen.
b) A low magnification micrograph of 6 h post YHV-infected lymphoid tubule, showing large numbers of immature (Im), agranular (AGH), small granular (SGH) and few large granular (LGH) haemocytes fully packed the lumen as well as migrating into the wall of the lymphoid tubule.
c) A medium magnification micrograph of a large granular haemocyte infected with YHV at the early stage. Numerous unenveloped-YHV particles were observed in its peripheral cytoplasm (boxed area), where an inset showed high magnification of YHV particles.
d) A medium magnification micrograph of a stromal cell infected with YHV at the late stage. Enveloped-YHV particles were observed in its peripheral cytoplasm, where an inset of the boxed area showed high magnification of YHV particles.

membrane (Figure 3b) at 18 h PI. Later viral particles were uncoated in the endosome and released into the cytoplasm by the breaking down of endosomal membrane, thus leaving emptied endosomal vesicles (Figure 3c). At 24-36 h PI, there were long filamentous unenveloped YHV scattering throughout the cytoplasm (Figure 3d), where later the uncoated particles rapidly increased in number. Most coated viral particles were found in Golgi complexes (Figure 3e) and rough endoplasmic reticulum (Figure 3f); thus YHV might undergo maturation and budding in the RER-Golgi system. At 48-60 h PI, a large number of completely enveloped viruses was tightly packed within membrane-bound vesicles underneath the cell membrane (Figure 4a, 4b). These vesicles were later fused with the cell membrane (Figure 4c) and released

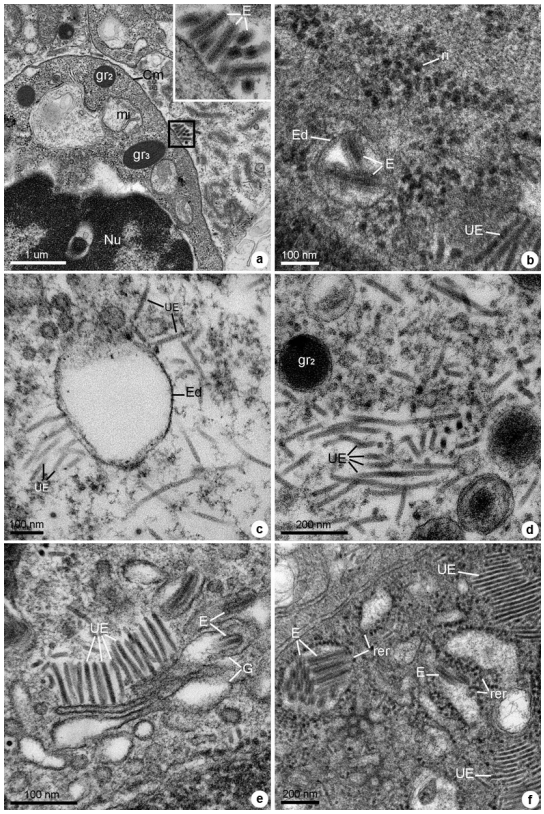

Figure 3. TEM micrographs of the viral distribution in lymphoid cells during early stages (18-36 h) after YHV infection.

a) A medium magnification micrograph of a granular haemocyte at 18 h post infection (PI), showing fully enveloped YHV particles attached to the cell membrane (Cm) at the boxed area. These granular haemocytes could be identified by their distinctive granules, i.e., gr_2 and gr_3, respectively. The inset shows high magnification of the boxed area that exhibited fully enveloped viral particles.

b) A high magnification micrograph of a stromal cell at 18 h PI, showing penetration step in which enveloped (E) YHV particles were present in a membrane-bounded endosomal vesicle (Ed). ri-ribosome, UE-unenveloped YHV particle.

c) A high magnification micrograph of a stromal cell at 18 h PI, showing uncoating step in which unenveloped (UE) YHV particles were released from the empty and broken up endosomal vesicle (Ed).

d) A high magnification micrograph of a granular haemocyte at 24 h PI, showing replication step in which numerous long filamentous unenveloped (UE) YHV particles or nucleocapsids were found throughout the cytoplasm of the haemocyte. gr_2-granule type 2

e, f) High magnification micrographs of a stromal cell at 36 h PI, showing early maturation step in which few enveloped (E) of YHV particles were found in Golgi complex (G) (e) and rough endoplasmic reticulum (RER) (f).

complete viral particles to the exterior by exocytosis (Figure 4d). At 72 h PI, the entire cell cytoplasm was fully packed with numerous unenveloped viruses, while the enveloped viruses were within the membrane-bounded vesicles (Figure 4e), giving the impression of severe infection. In such case, the complete viral particles as well as unenveloped viruses were released due to the general breakdown of the cells (Figure 4f).

4. Discussion

Viral particles were detected in stromal matrix cells and lymphoid tubule haemocytes (LTH). The pattern of viral particle localisations was similar in both cells. The fully enveloped viral particles were detected at the cell membrane, endosomal vesicles, Golgi complex, large and dilated sacs of RER, and in exocytotic vesicles, whereas the unenveloped (uncoated) nucleocapsids could be detected in the cytoplasm, but not in the area underneath the cell membrane. The distributions of coated and uncoated yellow head viral particles in these specific areas and organelles of the cells were similar to the other positive-stranded RNA viruses such as severe acute respiratory syndrome (SARS), corona viruses (Stadler *et al*., 2003), and hepatitis C virus (Kaito *et al*., 2006). These observations suggest that the putative pathway for YHV in stromal and LTH cells (Figure 5) is as follows; YHV first makes contact with the cell membrane and being lipid in nature the viral envelope could easily fuse with the cell membrane. This process could also be mediated by fusogenic proteins, such as spikes present on the envelope (Spaan *et al*., 1988; Assavalapsakul *et al*., 2005). The entry is probably mediated by endocytotic pathway as no free viral particles were detected under the cell membrane. Instead, there were a few endosomal-liked vesicles containing both fully enveloped and uncoated viral particles. The viruses were probably uncoated in the endosome complex and released into the cytoplasm by the breaking down of endosomal membrane (Figure 3c). Once the uncoated nucleocapsids were in the cytoplasm the single positive strand viral RNA probably underwent both translation and replication. The actual details are not known; however, based on similar viruses that contain single positive RNA strand, such as SARS and corona virus (Ng *et al*., 2003), it is plausible that positive RNA strand of YHV might be used as the template for constructing negative RNA strand, which would in turn act as the template for a new positive strand. Further templating and replication could produce multiple copies of positive RNA strands that could also act as mRNA for the translation of capsid proteins on the non-membrane bound ribosomes of the infected cells. The whole process of the RNA replication and capsid protein synthesis was most likely taking place in the cytoplasm as no viral particles were detected in the nucleus as previously reported (Chantanachookin *et al*., 1993; Flegel *et al*., 1995).

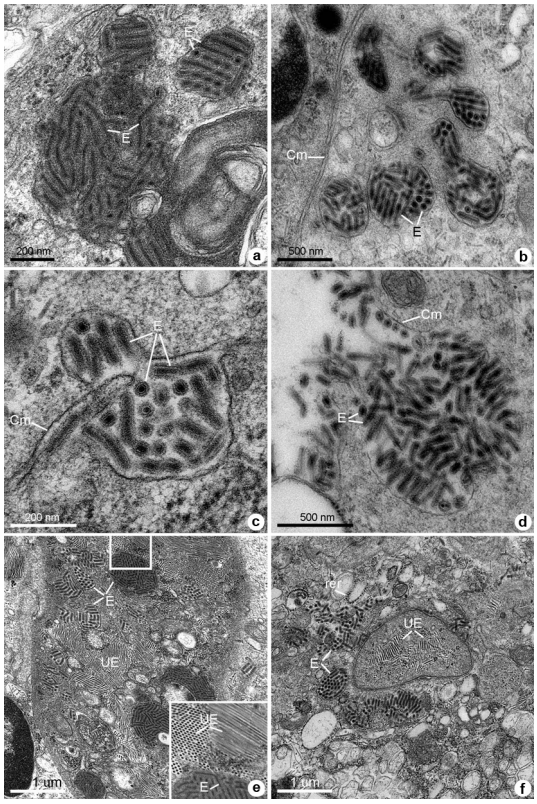

Figure 4. TEM micrographs of the viral distribution in lymphoid cells during late stages (48-72 h) after YHV infection.
a) A high magnification micrograph of a stromal cell at 48h PI, showing late maturation step in which numerous enveloped YHV particles (E) were contained within membrane-bound vesicles.
b-d) High magnification micrographs of stromal cells at 48h PI, showing releasing step in which there were membrane-bound vesicles containing tightly packed mature and fully enveloped YHV particles (E) close to cell membrane (Cm) (b); these vesicles fused with the membrane (c and d) to release YHV particles by exocytosis (d).
e, f) Medium magnification micrographs of stromal cells at 72 h PI, showing severely infected stromal cells whose cytoplasm were fully packed with numerous YHV particles (e) that eventually burst out (f). Enveloped particles (E) were packed in membrane-bound vesicles, whereas unenveloped particles (UE) were scattered throughout the cytoplasm (e, inset).

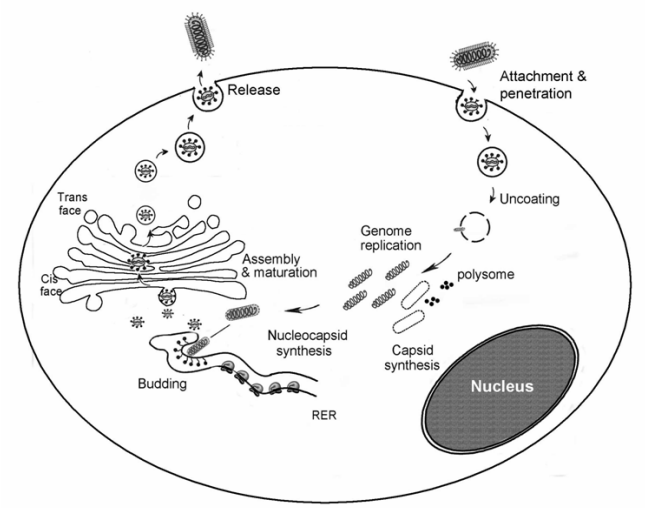

Figure 5. A schematic diagram illustrating the putative pathway for YHV infection in stromal cells and haemocytes in the lymphoid tubule wall. The viral particles enter the cells by endocytosis, become uncoated, pass into the cytoplasm where the viral genomes are replicated, and nucleocapsid proteins synthesized. The nucleocapsids with genomes bud into rough endoplasmic reticulum (RER) and are transferred through RER-Golgi compartment where the envelopes are formed. Eventually, the completely enveloped viral particles are packaged in secretory vesicles and released by exocytosis at the cell membrane.

It is likely that only positive RNA strands are self-assembled with capsid proteins in the cytoplasm (den Boon, *et al.,* 2010). TEM observation did show a high concentration of uncoated YHV nucleocapsids in the cytoplasm of stromal and LTH cells. These particles were tightly packed but were never found inside vesicles or surrounded by any membrane (Figure 3d). On the contrary, the large aggregates of completely coated YHV particles were always packed inside membrane-bounded compartments or large vesicles, comprising Golgi complexes (Figure 3e), dilated RER (Figure 3f) and large secretory vesicles (Figure 4a-e). Therefore, we suggest that from the cytoplasm the nucleocapsids budded into the membrane of RER, where the spike proteins were synthesized from the whole positive RNA strand template or parts of it on the RER-bound ribosomes. Once these spike proteins were made they intercalated themselves into the lipid of RER and Golgi complex membrane, much like the synthesis and insertion of integral plasma membrane proteins of mammalian cells as reported by many studies (MacKinnon, 2005; Lee *et al*., 2004). The budding of YHV nucleocapsids into the membrane of RER and Golgi complexes, which already bore spike proteins, could result in the formation of completely enveloped YHV. These mature viral particles could then be transported into large secretory vesicles derived from the Golgi complexes. Finally, these large secretory vesicles joined up with the plasma membrane and released the mature YHV by exocytosis (Figure 4b-d). Alternatively, if the cells were highly infected they could succumb and undergo necrosis and literally burst out and release both coated and uncoated YHV particles through exocytosis and general cellular break down.

Acknowledgements

This research was supported by Office of Higher Education Commission and Mahidol University under the

National Research Universities Initiative. We would also like to thank Ms. Jaruwan Poljareon for assistance with the graphics.

References

Anantasomboon, G., Poonkhum, R., Sittidilokratna, N., Flegel, T.W. and Withyachumnarnkul, B. 2008. Low viral loads and lymphoid organ spheroids are associated with yellow head virus (YHV) tolerance in whiteleg shrimp *Penaeus vannamei*. Developmental & Comparative Immunology. 32, 613-626.

Anggraeni, M.S. and Owens, L. 2000. The haemocytic origin of lymphoid organ spheroid cells in the penaeid prawn *Penaeus monodon*. Disease of Aquatic Organisms. 40, 85-92.

Assavalapsakul, W., Tirasobhon, W. and Panyim, S. 2005. Antiserum to the gp116 glycoprotein of yellow head virus neutralizes infectivity in primary lymphoid organ cells of *Penaeus monodon*. Disease of Aquatic Organisms. 63, 85-88.

Boonyaratpalin, S., Supamattaya, K., Kasornchandra, J., Direkbusarocom, S., Aekpanithanpong, U. and Chantanachooklin, C. 1993. Non-occluded baculo-like virus, the causative agent of yellow head disease in the black tiger shrimp (*Penaeus monodon*). Gyobyo Kenkyu. 28, 103 - 109.

Chantanachookin, C., Boonyaratpalin, S., Kasornchandra, J., Direkbusarakom, S., Ekpanithanpong, U., Supamataya, K., Sriuratana, S. and Flegel, T.W. 1993. Histology and ultrastructure reveal a new granulosis-like virus in *Penaeus monodon* affected by yellow- head disease. Disease of Aquatic Organisms. 17, 145-157.

Cowley, J.A., Dimmock, C.M., Wongteerasupaya, C., Boonsaeng, V., Panyim, S. and Walker, P.J. 2000. Gill-associated virus of *Penaeus monodon* prawns: an invertebrate nidovirus with ORF1a and ORF1b genes related to arteri- and coronaviruses. Journal of General Virology. 81, 1473-1484.

den Boon, J.A., Diaz, A., and Ahlquist, P. 2010. Cytoplasmic Viral Replication Complexes. Cell host & Microbe. 8, 77-85.

Duangsuwan, P., Phoungpetchara, I., Tinikul, Y., Poljaroen, J., Wanichanon, C. and Sobhon, P. 2008. Histological and three dimensional organizations of lymphoid tubules in normal lymphoid organ of *Penaeus monodon*. Fish and Shellfish Immunology. 24, 426-435.

Duangsuwan, P., Tinikul, Y., Chotwiwatthanakun, C., Vanichviriyakit, R. and Sobhon, P. 2008. Changes in the histological organization and spheroid formation in lymphoid organ of *Penaeus monodon* infected with yellow head virus. Fish and Shellfish Immunology. 25, 560-569

Flegel, T.W., Sriurairatana, S., Wongteerasupaya, C., Boonsaeng, V., Panyim, V. and Withyachumnarnkul, B. 1995. Progress in characterization and control of yellow-head virus of *Penaeus monodon*. In: Browdy CL, Hopkins JS (eds) Proceedings of the Special Session on Shrimp Farming, Aquaculture '95, San Diego, February 1995. World Aquaculture Society, Boston Rouge, FL, pp. 76-83.

Flegel, T.W. 2006. Detection of major penaeid shrimp viruses in Asia, a historical perspective with emphasis on Thailand. Aquaculture 258, 1-33.

Hasson, K.W., Lightner, D.V., Mohney, L.L., Redman, R.M. and White, B.M. 1999. Role of lymphoid organ spheroids in chronic Taura syndrome virus (TSV) infections in *Penaeus vannamei*. Disease of Aquatic Organisms. 38, 93-105.

Kaito, M., Ishida, S., Tanaka, H., Horiike, S., Fujita, N., Adachi, Y., Kohara, M., Konishi, M. and Watanabe, S. 2006. Morphology of hepatitis C and hepatitis B virus particles as detected by immunogold electron microscopy. Medical Molecular Morphology. 39, 63-71.

Kanobdee, K., Soowannayan, C., Flegel, T.W., Ubol, S., and Withyachumnarnkul, B. 2002. Evidence for apoptosis correlated with mortility in the giant black tiger shrimp *Penaeus monodon* infected with yellow head virus. Disease of Aquatic Organisms. 48, 79-90.

Kasornchandra, J. and Boonyaratpalin, S. 1995. Electron microscopic observation on the replication of yellow head baculovirus in the lymphoid organ of *Penaeus monodon*. In: Shariff M, Arthur JR, Subasinghe RP (eds) Disease in Asian Aquaculture II. Manila: Fish Health Section, Asian Fisheries Society, pp. 99-105.

Lee, M.C.S., Miller, E.A., Goldberg, J., Orci, L. and Schekman, R. 2004. Bi-directional protein transport between the ER and Golgi. Annual Review of Cell and Developmental Biology. 20, 87-123.

Lu, Y., Tapay, L.M., Loh, P.C. and Brock, J.A. 1994. Infection of the yellow head baculo-like virus (YHB) in two species of penaeid shrimp, *Penaeus stylirostris* (Stimpson) and *Penaeus vannamei* (Boone). Journal of Fish Diseases. 17, 649-656.

MacKinnon, R. 2005. Structural biology: membrane protein insertion and stability. Science 307, 1425-1426.

Munro, J. and Owens, L. 2007. Yellow head-like viruses affecting the penaeid aquaculture industry: a review. Aquaculture Research. 38, 893-908.

Nadala, E.C.B., Tapay, L.M. and Loh, P.C. 1997. Yellow-head virus: a rhabdovirus-like pathogen of penaeid shrimp. Disease of Aquatic Organisms. 31, 141-146.

Ng, M.L., Tan, S.H., See, E.E., Ooi, E.E. and Ling, A.E. 2003. Proliferative growth of SARS coronavirus in Vero E6 cells. Journal of General Virology. 84, 3291-3303.

Paterson, W.D. and Stewart, J.E. 1974. *In vitro* phagocytosis by hemocytes of American lobster *(Homarus americanus)*. Journal of the Fisheries Research Board of Canada. 31, 1051-1056.

Rusaini and Owens, L. 2010. Insight into the lymphoid organ of penaeid prawns: A review. Fish and Shellfish Immunology. 29, 367-377

Schwartz, M., Chen, J., Lee, W.M., Janda, M. and Ahlquist, P. 2004. Alternate, virus-induced membrane rearrangements support positive-strand RNA virus genome replication. Proceedings of the National Academy of Sciences. 101, 11263-11268.

Sittidilokratna, N., Hodgson, R.A.J., Panyim, S. and Cowley, J.A., Jitrapakdee, S., Boonsaeng, V., Walker, P.J. 2002. The complete ORF1b-gene sequence indicates yellow head virus is an invertebrate nidovirus. Disease of Aquatic Organisms. 50, 87-93.

Spaan, W., Cavanagh, D. and Horzinek, M.C. 1988. Coronavirus structure and genome expression. Journal of General Virology. 69, 2939-2952.

Spann, K.M., Cowley, J.A., Walker, P.J. and Lester, R.J.G. 1997. A yellow-head-like virus from *Penaeus monodon* cultured in Australia. Disease of Aquatic Organisms. 31, 169-179.

Stadler, K., Masignani, V., Eickmann, M., Becker, S., Abrignani, S., Klenk, H.D. and Rappuoli, R. 2003. SARS-beginning to understand a new virus. Nature Reviews Microbiology 1(3), 209-218.

Soowannayan, C., Sithigorngul, P. and Flegel, T.W. 2002. Use of a specific monoclonal antibody to determine tissue tropism of yellow head virus (YHV) of *Penaeus monodon* by *in situ* immunocytochemistry. Fisheries Science. 68 (Suppl I), 805-809.

Van de Braak, C.B.T., Faber, R. and Boon, J.H. 1996. Cellular and humoral characteristics of *Penaeus monodon* (Fabricius, 1798) haemolymph. Comparative haematology international. 6, 194-203.

Walker, P.J., Sittidilokratna, N., Mahy, B.W.J. and Regenmortel, M.H.V. 2008. Yellow Head Virus, Encyclopedia of Virology, Academic Press, Oxford, England, pp 476-483.

Wang, Y.C. and Chang, P.S. 2000. Yellow head virus infection in the giant tiger prawn *Penaeus monodon* cultured in Taiwan. Fish Pathology. 35, 1-10.

Wongteerasupaya, C., Sriurairatana, S., Vickers, J.E., Akrajamon, A., Boonsaeng, V., Panyim, S., Tassanakajon, A., Withyachumnarnkul, B. and Flegel, T.W. 1995. Yellow head-virus of *Penaeus monodon* is an RNA virus. Disease of Aquatic Organisms. 21, 69-77.