計畫編號：BM04-00
計畫名稱:蝦及其它動物模式中病毒與宿主間交互作用之整合生物學
計畫主持人：羅竹芳
計畫摘要(中)：

病毒有兩個十分重要的策略性目標: 其一是達成感染以及病毒複製過程中之重要步驟，另一方面則是同時對抗宿主發展出對抗病毒而發展出的特殊細胞防禦反應。現今科學界仍無法全盤瞭解病毒對抗宿主的機制，宿主對抗外來入侵者的策略也仍有相當大的研究空間。因此本整合型計畫著眼於此國際性重要及競爭之科學領域，進行動物對疾病反應的系統生物學研究，其中將以蝦類/病毒之交互作用為研究主軸，至於其他模式生物與其病毒的交互作用之研究則可做為借鏡與比較。

台灣大學生命科學院同仁長期致力於模式生物 (例如線蟲，果蠅，斑馬魚) 以及經濟生物 (蝦及養殖魚) 的功能基因體學研究。大多數研究團隊成員在前二年的時間中，合作參與了第一期臺灣大學拔尖計畫。在該合作計畫 “病毒與動物宿主間交互作用之功能基因體學研究” 中，本研究團隊建立了有力的技術平台，並成功擴展蝦類病毒性疾病以及病毒與宿主間交互作用相關的知識。本計畫承接並延伸先前的研究成果，並著眼於病毒 (蝦白點病毒) 感染重要經濟水生甲殼動物後，宿主及病毒之間的對抗及反制策略之研究。另一方面，二個重要模式生物 (斑馬魚/神經壞死病毒以及轉染蝦白點病毒之線蟲) 的病毒感染研究也會同時進行，以做為比較(水生脊椎動物與無脊椎動物之對照) 及由分子細胞層次上詮釋致病機制(轉染蝦白點病毒之線蟲系統)。總結而言，本群體計畫將有七個具有互補性的子計畫，使用各種先進的技術和科學新知來探討宿主受到病毒感染時二者間的互動關係。

所有子計畫評估將由總主持人以及評估委員執行。本計畫中的子計畫都由具世界競爭力的學者執行，並著眼於世界級的重要研究領域(病毒/宿主交互作用)。我們預期本計畫的成功將可提升台灣大學在科學研究領域的重要性及競爭力。

計畫摘要(英)：

Viruses have two fundamental strategic objectives: on the one hand they must accomplish the steps necessary for infection and replication,

and meanwhile they must simultaneously overcome the specific cellular defenses that are deployed against them by the host. Science is very far from understanding the full range of mechanisms that are used by the virus to thwart the host, and equally far from understanding the mechanisms by which the host attempts to thwart the invader. This proposal is for a coordinated project that focuses on this internationally important and competitive area of science. The seven principal investigators in this project will systematically study animal disease responses, particularly the host/virus interactions in a viral pathogen/shrimp host system as well as in other animal models.

The research team for this project was assembled by NTU's College of Life Science, and it has considerable experience in using functional genomics approaches for basic science research on model organisms (Caenorhabditis elegans, zebrafish) and on other economically important species (shrimp and cultured fish). Most of the team members have already worked together on the first two-year, phase I part of this Excellent Research Project of National Taiwan University. The phase I project was "an integrated functional genomics study of host/virus interaction in a shrimp host system and in other animal models", and while working together, the team established very strong technological platforms that were successfully used to increase knowledge of shrimp viral diseases, and of virus/host interactions. The present proposal therefore aims to continue and extend these earlier studies by focusing on the strategies and counter-strategies that are used during a viral (white spot syndrome virus) infection of an economically important crustacean. Viral infection in two other animal models (zebrafish/nervous necrosis viruse and WSSV-transfected C elegans) for the purpose of comparison ((zebrafish system; aquatic vertebrate vs invertebrate) and to elucidate the mechanisms at the cellular and molecular levels (WSSV-transfected C elegans system). In total, there will be seven complementary projects that will use various state-of-the-art techniques and scientific understanding to help formulate a big picture of the strategies and counter strategies that are used during a virus infection of a crustacean host.

Evaluation of all the subprojects will be conducted by the Chief

Investigator, and her evaluating committee. The work of all seven subprojects will be conducted by world class scientists and will focus on a world-class area of study. We anticipate that success in this project will put NTU at the forefront of an internationally important and competitive area of science.

計畫編號：BM04-01
計畫名稱:蝦白點病毒反擊寄主防禦反應並成功在細胞中複製的策略
計畫主持人：羅竹芳
計畫摘要(中)：

白點症病毒為一具有高侵襲性之蝦類致病原，其所造成的蝦類白點症嚴重衝擊了包含我國在內許多國家的蝦類養殖產業。白點症病毒為 Nimaviridea 病毒科、Whispovirus 屬之模式種病毒，白點症病毒為一大型 DNA 病毒，其病毒顆粒由核蛋白鞘(nucleocapsid)，膜外衣(tegument)及套膜(envenlop)所組成。此病毒非常獨特，其感染策略與其他已知病毒皆不相同，因此須對此進行嶄新的研究。 儘管如此，白點症病毒仍需在功能上滿足與其他病毒成功感染所需的條件，也就是此種獨特性，使其成為趨同及趨異演化事實也將被揭露。目前已對三個白點症病毒分離株的基因體完成定序，此外，藉由三個最大的蝦類 EST 研究計畫，共計約有四萬多筆蝦類基因序列被發表(由多至少，分別為 15981 筆, 13656 筆及 10100 筆)，這些成果，使得我們得以利用基因體學及蛋白質體學的研究方法對此病毒及其與寄主之間的交互作用，進行分子層次的總體特性分析，這些分析方法的可行性已被成功驗證並藉此發現許多重要的白點症病毒基因，例如病毒極早期基因、病毒構造性蛋白及許多具有重要功能的非構造性蛋白。

此病毒最具代表性的寄主為具高經濟效益的美洲白蝦及草蝦，目前對蝦類寄主的研究主要著重於蝦類抗病毒機制，研究發現白點症病毒感染會調控許多寄主基因的表現，寄主基因表現形態的改變，不只反映出蝦類寄主對抗病毒感染的反應，也暗示著白點症病毒擊潰寄主細胞正常功能以利其增殖的可能機制。已知受病毒感染細胞常藉由細胞凋亡反應以限制病毒增殖，雖然蝦類誘發細胞凋亡作用的因子並不清楚，但我們發現受白點症病毒感染的細胞其細胞凋亡作用會被抑制。白點症病毒 WSSV449 的基因產物可直接抑制蝦類寄主 Caspase 的活性，以達到阻斷細胞凋亡反應的進行。此外，已知許多訊息傳遞路徑在寄主免疫反應中扮演重要的角色，其中 JAK/ STAT 訊息傳遞

路徑常被活化以誘發寄主的抗病毒反應，但白點症病毒卻會利用活化的 JAK/ STAT 訊息傳遞路徑以增進其極早期基因的表現。

白點症病毒致病力極高，具有廣寄主域並會侵襲多種組織，白點症病毒的複製常被生理及環境的緊迫所誘發，而白點症病毒於甲殼類寄主體內快速增殖的特性也暗示著其可成功擊潰、克服或適應寄主的抗病毒機制。儘管藉由白點症病毒功能性基因體學的研究已提供許多白點症病毒及其與寄主交互作用的資訊，但仍對此病毒的感染策略及寄主的抗病毒機制欠缺全盤性的了解，因此，本分項計畫的目標在於延續之前的研究，以釐清寄主防禦機制及參與在擊潰、克服或適應寄主防禦機制之病毒相對應機制。

計畫摘要(英)：

White spot syndrome virus (WSSV) is the causative agent of a disease that has led to severe mortalities of cultured shrimps in Taiwan and many other countries. WSSV is the type species of the genus Whispovirus, family Nimaviridae. WSSV is a large DNA virus with a virion that consists of a nucleocapsid, tegument and envelope. WSSV is very unique, with an infection strategy that does not match the infection models of any other known virus, and it must therefore be investigated ab initio. Functionally, however, WSSV must fulfill many similar requirements to other viruses; thus its uniqueness makes it very likely that instances of convergent and divergent evolution will be identified. Meanwhile, the genomes of 3 WSSV isolates have already been completely sequenced, and currently, the three largest penaeid shrimp EST studies have published 15,981, 13,656 and 10,100 ESTs. This allows us to use genomic and proteomic approaches to effect a global molecular characterization of the virus and its interactions with the host. These approaches have proved very successful and have recently led to the discovery of many important WSSV genes, including immediate early genes, structural genes and many other non-structural genes with important functions. The host animals are represented by Penaeus vannamei and P. monodon, both of which are economically important. Much of our current work focuses on the strategies and counter-strategies that are deployed during a viral infection of Shrimp. We show that WSSV infection modulates expression of various kinds of genes. These gene

expression pattern changes not only reflect the responses of shrimp to the virus infection but also suggest how WSSV subverts cellular functions for virus multiplication. Blocking apoptosis in infected cells can facilitate multiplication of the virus, and we have found that WSSV acts to prevent apoptosis in infected cells. Although the factors that induce the apoptosis are currently unknown, a WSSV apoptosis protein (ORF449) was recently characterized. Further studies have shown that the WSSV anti-apoptosis protein is a direct P. monodon caspase inhibitor. We also found that WSSV actually co-opts a signaling pathway (the JAK/STAT signaling pathway) that is normally induced as an antiviral response in order to regulate immediate early gene expression.

WSSV is extremely virulent, having a wide host range and targets various tissues. Replication of the virus is easily triggered by physiological or environmental stress, and the rapid replication of WSSV inside crustacean hosts suggests that it has evolved mechanisms that successfully avoid, neutralize or subvert the various anti-viral, immune response defense mechanisms used by the host. Although our previous studies on functional genomics of WSSV have so far provided some information about the interactions of WSSV and host, we are still very far from understanding the full range of mechanisms that are used by WSSV to thwart the shrimp, and equally far from understanding the mechanisms by which the shrimp attempts to thwart the invader. Our objective in this sub-project is therefore to continue our previous study to identify the defense mechanisms of the host and the counter-mechanisms which the virus has evolved to defeat/circumvent/co-opt these host defenses.

計畫編號：BM04-02

計畫名稱：分析蝦白點病毒蛋白質體交互作用網絡及其誘發逆境反應之機制

計畫主持人：黃偉邦

計畫摘要(中)：

蝦類水產養殖是亞洲地區的重要產業項目之一，但近年來此相關產業持續不斷受到幾種蝦類病毒性疾病的危害，其中又以白點症病毒(white spot syndrome virus, WSSV)所引起的白點症(white spot syndrome)所造成的損失最為嚴重。然而，僅管面對此一嚴峻的挑戰

與過去對此病毒的研究之努力，如今我們仍然缺乏控制白點症疾病之擴散與治療的有效方法，究其原因多肇因於白點症病毒之獨特性。白點症病毒之基因體約 300 kb 長，已在數年前完成解序，依基因體資訊推測，白點症病毒最多可能帶有約 500 個開放轉譯區(open reading frame)，其中大部份的開放轉譯區序列與其他病毒或物種的基因缺乏明顯的相似性，因此增加了對白點症病毒研究的困難度。有鑑於此，本計畫之目標以建立白點症病毒基因交互作用(interactome)網路為出發點，我們計畫利用酵母菌雙雜合實驗方法對白點症病毒的主要基因進行分析，在建立此資訊後，我們將分析尋找適當的白點症病毒基因產物，進而以其為標的分析蝦類宿主細胞可以與之作用的蛋白質，期望由此可以啟始分析白點症病毒感染宿主細胞過程中的重要交互作用，進而由這些資訊分析白點症病毒在感染宿主過程中，誘發宿主細胞產生細胞自噬與計畫性細胞死亡等逆境反應的機制，最終希望分析未來經由調控宿主細胞逆境反應以對抗白點症疾病之可能性。

計畫摘要(英)：

White spot syndrome virus (WSSV) infection results in white spot disease in crustaceans and is causing considerable economic losses in shrimp farming industry worldwide. Despite of its stern condition and past research efforts desiring to find cures for the disease, an effective vaccine or curing medicine is still missing. Therefore, studying the interaction between WSSV and host cells will not only broaden our knowledge on this virus but also help us find effective regimens for this disease. The around 300-kb genome of WSSV encodes more than 500 putative open reading frames. Some of them have been characterized encoding structural proteins of WSSV virions and many others are expressed during virus infection. These WSSV proteins are hence good candidates for developing strategy to control white spot disease and construction of their interaction partnership is the first step marching toward that goal.

The objectives of this sub-project are to establish the interaction network maps of viral proteins. Based on this information, the crucial candidate proteins for WSSV infectious and pathological effects will be identified by analyzing those proteins with most interaction partners. These candidate proteins will be further applied for screening their

interaction partners in host cells. Through this strategy, we plan to decipher the mechanisms for WSSV viral entrance into host cells and WSSV replication control. In addition, viral infection is known to elicit multiple stress responses in host cells, including apoptosis and autophagy. These stress responses are related to crustacean innate immunity, which operate to minimize the deliberate effects of viral infection. These responses, however, may lead to individual shrimp death in hope to limit the production of new virions. Induction of autophagy during infection, on the other hand, is known to be essential for some virus replication in host cells. Therefore, the role of autophagy in virus control is still on debate. Current views on the autophagy pathway suggest that autophagic degradation within limited level is beneficial to cells for eliminating damaging agents, such as intracellular pathogens and protein aggregates. Overly activated autophagy activity will then cause cell death due to the elimination of components essential to maintain cell life. How virus cope these multiple cellular response mechanisms for their benefits remains largely unknown. Recently, one open reading frame of WSSV genome was found encoding an anti-apoptotic protein, suggesting that WSSV is capable of control host cell apoptotic pathway. If WSSV also regulates autophagy, on the other hand, has not been reported yet. We plan to study autophagy regulation by WSSV during infection. With the WSSV and host protein interaction information in hands, we will further characterize those interactions between viral proteins and host proteins related to stress responses. The abilities for WSSV proteins to regulate autophagy will be examined in both Saccharomyces cerevisiae model organism and Penaeus monodon. With future aqua cultural applications in our mind, whether manipulation of stress response activities of shrimp host cells may modulate viral infection will be tested and their possibilities for clinical application will be analyzed.

計畫編號：BM04-03

計畫名稱：蝦的 Sp1 蛋白質調控蝦白點病毒極早期基因表現之機轉

計畫主持人：張麗冠

計畫摘要(中)：

蝦白點病毒（WSSV）是一個大的含有外套膜的雙股圓形 DNA

病毒，基因組約有 300 kb，對蝦的致死率高達 90-100%。由於缺乏蝦的細胞株，對於蝦白點病毒基因表現的調控機轉目前大都不甚瞭解。計畫總主持人羅教授的研究室首先利用 cycloheximide 處理蝦，然後以 microarray 及 RT-PCR 的技術找到三個蝦白點病毒的極早期基因 ie1, ie2 及 ie3。其中 ie1 基因的啟動子在昆蟲 Sf9 細胞中具有很高的表現，可能是因為 STAT 結合在 ie1 基因的啟動子上所造成的。本研究計畫主要的目的是要探討蝦白點病毒的極早期基因之調控機制。首先由序列分析發現在 ie1 啟動子上游-102 到-97 及 ie2 啟動子上游-352 到-347 的區域含有 Sp1 結合序列。另外也發現在蝦（Penaeus monodon）的基因庫中有一個 EST 序列（HPA-N-S01-EST0038），與斑馬魚的 Sp1 有同源序列，而斑馬魚的 Sp1 蛋白質序列和人類的 Sp1 蛋白質在 DNA 結合區域有極高的相似度，顯示不同來源的 Sp1 蛋白質可能都會結合在類似的 DNA 序列上。目前已知，人類的 Sp1 是一種會結合在很多 GC 序列（GC boxes）的廣泛存在且多功能的蛋白質，會影響細胞週期及末端分化等細胞功能。而 Sp1 的辨識序列在許多細胞與病毒基因的啟動子上都可以找到。 Sp1 也會藉由與 TAFII130 結合而活化沒有 TATA 序列的啟動子。而 ie2 與 ie3 的啟動子並不含 TATA 序列，因此 Sp1 很可能對 ie2 及 ie3 的轉錄作用是必要的。本研究目標是要（1）選殖與分析 P. monodon 的 Sp1 蛋白質；（2）利用昆蟲細胞證明蝦的 Sp1 對蝦白點病毒極早期基因 ie1 與 ie2 的調控作用；（3）證明蝦的 Sp1 調控 ie2 的轉錄作用。本研究將會闡述蝦白點病毒的基因表現調控機制，並能發展新的策略以便能抑制蝦白點病毒感染蝦及在蝦的細胞內複製。

計畫摘要(英)：

White spot syndrome virus (WSSV) is a large enveloped virus with about 300 kb double-stranded circular DNA that kills shrimps with a mortality rate of up to 90-100%. Because the lack of a shrimp cell line, the molecular mechanism that regulates the transcription of WSSV genes remain largely unknown. Professor Lo’s laboratory first identified three immediate-early genes, ie1, ie2, and ie3, of WSSV by using microarray and RT-PCR analysis from cycloheximide-treated shrimps. Among these immediate-early genes, ie1 displays high transcription activity in Sf9 insect cells, which can be attributed to the binding of STAT to the ie1 promoter. The main goal of this study is to analyze the regulation of

immediate-early genes of WSSV. Sequence analysis reveals that two consensus Sp1-binding elements are present in the region from －102 to －97 and －352 to －347 in the ie1 and ie2 promoters, respectively. Moreover, one expressed sequence tag (EST, HPA-N-S01-EST0038) in shrimps, Penaeus monodon, is homologous to the zebra fish Sp1. Comparison of Sp1 amino acid sequence from zebra fish and H. sapiens revealed a high degree of sequence identify in the DNA-binding domain of the proteins, suggesting that Sp1 from different species bind to similar DNA sequences. As is well known, human Sp1 is a ubiquitous and versatile protein that binds to GC-rich recognition elements (GC boxes) to influence different cellular functions such as cell cycle progression and terminal differentiation. Additionally, Sp1 recognition elements are distributed widely in various promoters of cellular and viral genes. Sp1 also interacts with TAFII130 to initiate transcription of TATA-less promoters. The ie2 and ie3 promoters do not contain TATA sequences, therefore, Sp1 may be necessary for the transcription of ie2 and ie3. The aims of this research are to (1) clone and analyze the recombinant PmSp1 from P. monodon; (2) examine the effect of PmSp1 on the transcription of ie1 and ie2 of WSSV in insect cells; (3) demonstrate PmSp1 involves in the regulation of the ie2 gene. This investigation will elucidate the molecular mechanisms that regulate the expression of WSSV genes and develop new strategies to inhibit infection and replication of WSSV in P. monodon.

計畫編號：BM04-04

計畫名稱：篩選草蝦與病毒感染之相關基因並分析其鄰近區域之基因體序列

計畫主持人：于宏燦

計畫摘要(中)：

草蝦是全世界最重要的養殖蝦種，然而近年來草蝦產業飽受疫病侵襲，野生母族群數量亦有逐漸枯竭之虞。草蝦雖富經濟價值，然而學界於蝦體生長、生殖、免疫調控等方面累積之基礎生物學極少，對其基因體之瞭解更是有限。因此，本計畫擬以瞭解草蝦基因體為目標，由草蝦 fosmid 基因體庫(五倍涵覆率)中，篩選對蝦體生存或生殖過程中具重要影響之基因—尤其是與蝦體抗病、抗緊迫、及性別分化

相關者。此外，為瞭解草蝦之基因體，我們將採取「浮掠定序」的策略，基因體定序之範圍限於含標的基因之部分基因體區域。基因體序列建立初稿之後，我們將定序來自不同族群的個體（包括台灣、菲律賓、越南、澳洲、泰國東部、泰國西部、馬達加斯加等族群），以瞭解這些基因體區域在族群間之遺傳變異、並建立單核苷酸多型性(SNPs）圖譜。SNPs 圖譜將有助於鑑別基因體中受正或負向選汰的重要基因。經序列分析後，若發現某對偶基因之頻度過高（或過低）、遺傳歧異度過高（或過低）、族群分化度增加、或過強的聯鎖不平衡等現象時，即可推知此基因體區域曾受過選汰的影響。這些知識將有助於解決草蝦養殖產業中所遭遇之疫病問題，並期能有效地改善草蝦養殖之產能。

計畫摘要(英)：

The black tiger shrimp, Penaeus monodon, is the most important shrimp aquaculture species in the world especially in the Indo-Pacific region. The shrimp industry is now threatened by diseases and depletion of the wild broodstock. While past research efforts have been on shrimp pathogens, the information about the host P. monodon remains scanty. Basic knowledge of shrimp biology, particularly with regard to the control of growth, reproduction and the immune system, is limited and hampered by the lack of genome information. So far, a number of P. monodon EST projects have been undertaken, and only a small number of P. monodon genes derived from these EST data have been cloned and characterized. We therefore constructed a fosmid library with 5X genomic coverage to provide as a useful resource for positional cloning and physical mapping. With this fosmid library, we will focus on identifying genes that play key roles in important biological processes, such as those involved in stress response, immunity, or sex differentiation. The genes that show differential expression patterns after virus infection, various stress treatments (elevated water temperature, low dissolved oxygen or significant reductions in salinity levels), or between different sexes will be our first priorities to screen. The structure and organization of genes will be delineated, including the upstream and downstream regulatory regions. Next, we will take a glimpse of P. monodon genome by a skim sequencing strategy, i.e., obtaining sequence

information from a fraction of genome by sequencing a collection of fosmid clones, including those with target genes. Furthermore, we will adopt a population genetics approach to identify the genes or genomic regions subject to natural selection, as they usually are functionally important. We will embark on an initial survey of genetic variation across the genomic regions that have been completely sequenced by extensive sequencing samples from various genetically diverse populations, including Taiwan (TW), the Philippines (PN), Vietnam (VN), Australia (AUS), eastern Thailand (Th-E), western Thailand (Th-W)], and Madagascar (MG) populations. The single nucleotide polymorphism (SNP) maps developed will contribute to identify specific loci with signatures of natural selection, which include a skew in the allele frequency distribution (i.e., an excess of low and/or high frequency derived alleles), reduced or enhanced levels of genetic variation, increased levels of population differentiation, and elevated levels of linkage disequilibrium (LD) relative to neutral expectation. Genes with higher nucleotide diversity (π) may involve in immune response and those with low or even no diversity may be related to essential metabolic mechanisms or developmental processes. And alleles that show long-range LD against a background of short LD might imply the presence of a selective sweep. In summary, understanding P. monodon genome, especially the genetic components of key biological and commercially relevant traits, will help in fostering a genome-era strategy for world shrimp industry to resolve its disease problems and improve the yield and quality of the shrimps.