WIV Newsletter
No.16 MAY 2017

Chinese and American scientists discussed policies in response to emerging infectious diseases

On 17-19 May 2017, 2nd China-U.S. Workshop on the Challenges of Emerging Infections, Laboratory Safety and Global Health Security was co-organized by U.S. National Academy of Sciences, Chinese Academy of Sciences (CAS), Wuhan Institute of Virology (WIV), CAS and Hubei Society for Microbiology. More than 100 experts from China, the United States, Pakistan and the Republic of Kenya attended the workshop.

The opening ceremony was chair by Prof. Zhiming Yuan, the Director of Wuhan National Biosafety Level 4 (P4) Laboratory. Prof. Xinwen Chen, the Director General of WIV, delivered the opening address, in which he pointed out that as a qualified academic exchange activity, this workshop will definitely put forward new ideas for the research on emerging viral diseases control, biosafety laboratory and global health security. The workshop is divided into five academic sessions. Experts delivered 23 speeches in the workshop, and mainly discussed policies in response to emerging infectious diseases. Prof. Linda Saif (academician) from Ohio State University, Prof. David Relman (academician) from Stanford University, Prof. James LeDuc, the Director of Galveston National Laboratory, and Prof. George F. Gao (academician) from Institute of Microbiology, CAS attended the workshop as special guests.

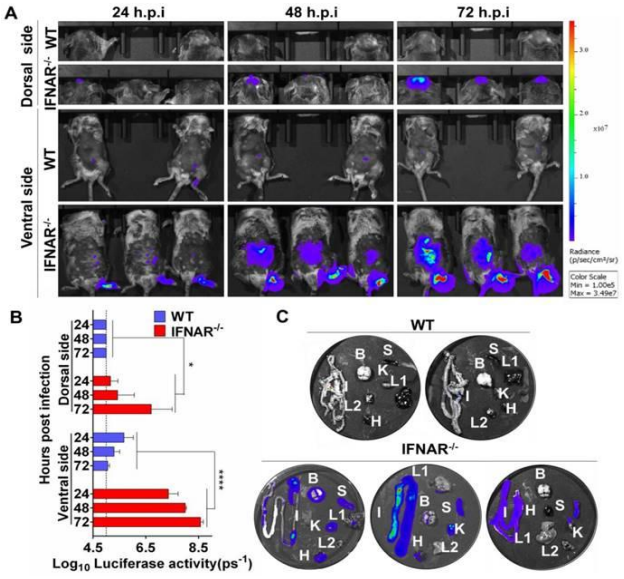
The 2nd China-U.S. Workshop on the Challenges of Emerging Infections, Laboratory Safety and Global Health Security belongs to the serial meetings jointly initiated by China and the United States. By gathering extraordinary reports, and discussing the current development on related studies, the participants can communicate and get further understanding regarding new issues that occur in field of global health biosafety. This workshop aims at utilizing the advantages of China-U.S. cooperation and collaboration for responding to emerging infectious diseases more positively and guarantee laboratory and global health biosafety, so as to make more contributions to the medical industry and human health.

Scientists reveal the pathogenic mechanism of arthropod-borne flaviviruses by using in vivo bioluminescence imaging technology

Flavivirus includes a large group of human pathogens with medical importance. Especially, neurotropic flaviviruses capable of invading central and peripheral nervous system, e.g. Japanese encephalitis virus (JEV) and Zika virus (ZIKV), are highly pathogenic to human and constitute major global health problems. However, the dynamic dissemination and pathogenesis of neurotropic flavivirus infections remain largely unknown.

In a present study, the research group led by Prof. ZHANG Bo from WIV, CAS and the research team led by Prof. QIN Chengfeng from Institute of Microbiology and Epidemiology of the Academy of Military Medical Sciences made an important breakthrough in the pathogenic mechanism of arthropod-borne flaviviruses. By using JEV as a model, they rationally designed and constructed a recombinant reporter virus that stably expressed Renilla luciferase (Rluc). The resulting JEV reporter virus (named Rluc-JEV) and parental JEV exhibited similar replication and infection characteristics, and the magnitude of Rluc activity correlated well with progeny viral production in vitro and in vivo.

By using in vivo bioluminescence imaging (BLI) technology, they dissected the replication and dissemination dynamics of JEV infection in mice upon different inoculation routes. Interestingly, besides replicating in mouse brain, Rluc-JEV predominantly invaded the abdominal organs in mice with typical viscerotropism. Further tests in mice deficient in type I interferon (IFN) receptors demonstrated robust and prolonged viral replication in the intestine, spleen, liver, kidney and other abdominal organs. Combined with histopathological and immunohistochemical results, the host type I IFN signaling was evidenced as the major barrier to the viscerotropism and pathogenicity of this neurotropic flavivirus.



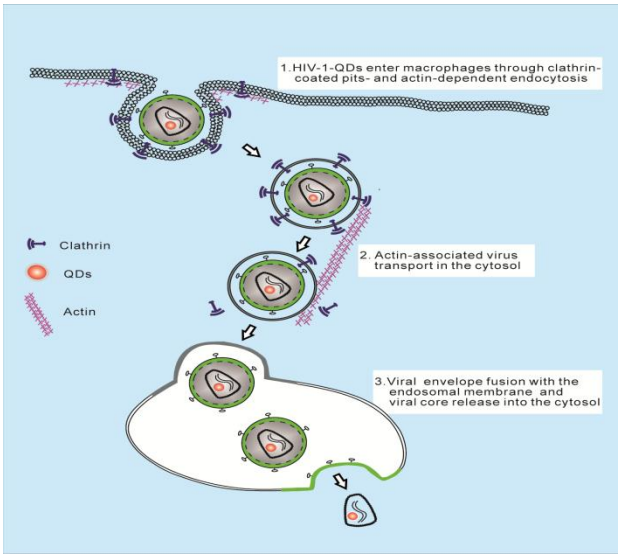
Additionally, the Rluc-JEV platform was readily adapted for efficacy assay of known antiviral compounds and a live JE vaccine. Collectively, their study revealed abdominal organs as important targets of JEV infection in mice and profiled the unique viscerotropism trait controlled by the host type I IFN signaling. This in vivo visualization technology described here provides a powerful tool for testing antiviral agents and vaccine candidates for flaviviral infection.

Link: *http://www.thno.org/v07p0912.htm*

Single-particle tracking reveals the mechanism for HIV-1 productive entry into primary macrophages

As one of the major targets of human immunodeficiency virus (HIV-1), macrophages (a type of white blood cell) play an important role in HIV infection. However, how the virus enters the macrophages is still poorly understood. In a present study, Prof. CUI Zongqiang from Wuhan Institute of Virology of the Chinese Academy of Sciences, Prof. ZHANG Xian'en from Institute of Biophysics of the Chinese Academy of Sciences, and Prof. WU Yuntao from George Mason University have now constructed a quantum dot (QD)-encapsulated infectious HIV-1 particle to observe the viral entry pathway to the macrophages at a single-particle level in live cells. The technique may assist in the development of inhibitors to block the HIV entry in macrophages.

Single-particle tracking is a powerful technique for studying viral entry, and inorganic semiconductor nanocrystal QDs are suitable for imaging single particles and tracking viral infection in live cells thanks to their remarkable brightness and the fact that they are photostable over time. The researchers have now used a technique called cell-assisted assembly to construct QD-encapsulated infectious HIV-1 particles that can be tracked in real time using florescence imaging. QDs were encapsulated in HIV-1 virions by incorporating viral accessory protein Vpr-conjugated QDs during virus assembly.



Prof. CUI and the colleagues monitored the early phase of viral infection in real time by using the HIV-1 particles encapsulating QDs. They observed that, HIV-1-QDs enter macrophages through clathrin-coated pits and actin-dependent endocytosis; endosome fusion is proved to be critical for HIV-1 productive infection in primary macrophages by a series of drug inhibition experiments. Additionally, they found that a dynamic actin cytoskeleton serves a vital role in HIV-1 entry in macrophages.

The researchers say that their findings may help to develop inhibitors to stop the HIV entry in macrophages, which may provide some new thoughts to the antiviral drugs development. These results have been published in *ACS Nano* entitled "Single-Particle Tracking of Human Immunodeficiency Virus Type 1 Productive Entry into Human Primary Macrophages". The research team also includes scientists from Huazhong University of Science and Technology in Wuhan.

Link: *http://pubs.acs.org/doi/abs/10.1021%2Facsnano.7b00275*

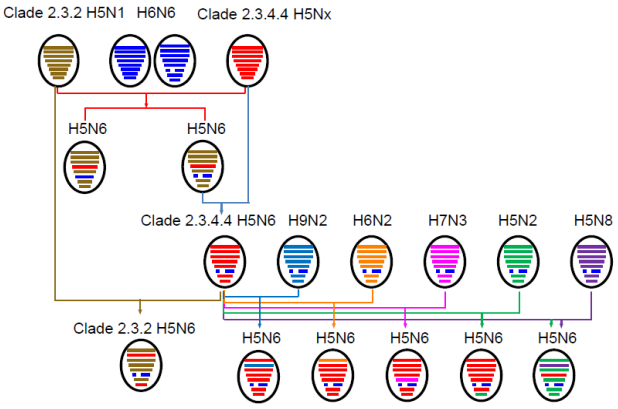
Researchers reveal a complex pattern of the evolution and emergence of H5N6 avian influenza virus in central China

H5N6 avian influenza virus (AIV) has posed a potential threat to public health since its emergence in China in 2013. To understand the evolution and emergence of H5N6 in the avian population, the research group led by Prof. CUI Jie from WIV, CAS developed the studies on the evolution and emergence of H5N6 avian influenza virus in central China. The scientists performed molecular surveillance of live poultry markets (LPMs) in Wugang prefecture, Hunan province, in central China during 2014-2015. Wugang prefecture is located on the Eastern Asian-Australian migratory bird flyway and a human death due to an H5N6 virus was reported in the prefecture on 21th November 2016.

In total, they sampled and sequenced the complete genomes of 175 H5N6 AIVs. Notably, our analysis revealed that H5N6 contains at least six genotypes arising from segment reassortment, including a rare variant that possesses an HA gene derived from H5N1 clade 2.3.2 and a novel NP gene that has its origins with H7N3 viruses. In addition, phylogenetic analysis revealed that genetically similar H5N6 AIVs tended to cluster according to their geographic region of origin.

These results help reveal the evolutionary behavior of influenza viruses prior to their emergence in humans.

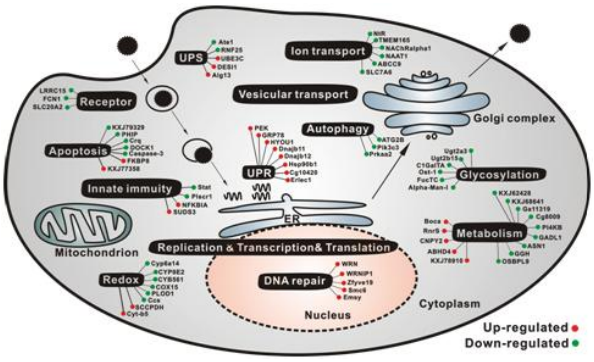
Link: *http://jvi.asm.org/content/early/2017/04/06/JVI.00143-17.abstract*



Scientists illustrate how host cell responds to Zika virus infection

Zika virus (ZIKV) is an emerging arthropod-born virus (arbovirus) belonging to the genus Flavivirus of the family Flaviviridae, which also includes a group of human pathogenic viruses such as dengue virus 1-4 (DENV 1-4), West Nile virus (WNV), Japanese encephalitis virus (JEV), yellow fever virus (YFV), and tick-borne encephalitis virus (TBEV). In early 2015, a ZIKV infection outbreak was recognized in northeast Brazil, which posed great threats to the human health. Many laboratories have screened the host factors that ZIKV replications rely on. However, questions regarding how host proteins are regulated during ZIKV infection at protein level remain unknown.

Mosquitoes are epidemiologically important vectors for ZIKV, and effective restrictions of ZIKV replication in mosquitoes will be effective in controlling the spread of



the virus. To identify proteins and pathways involved in ZIKV life cycles in mosquito cells, a quantitative proteomic analysis of ZIKV infected C6/36 cells was performed by the research group led by Prof. XIAO Gengfu from Wuhan Institute of Virology of the Chinese Academy of Sciences. The scientists quantified 3,544 host proteins, with 200 being differentially regulated. Bioinformatics analysis revealed that several ZIKV regulated biological processes. In addition, the further research indicated ubiquitin proteasome system (UPS) play roles in ZIKV entry process, and an FDA approved inhibitor of the UPS, Bortezomib, can inhibit ZIKV infection in vivo.

This is the first reported quantitative proteomic analysis of ZIKV infected host cells. To better understand the mosquito cell response to ZIKV infection, a map was created. In addition, this study provides a candidate drug for the control of ZIKV infection in mosquitoes and treatment of ZIKV infection in patient.

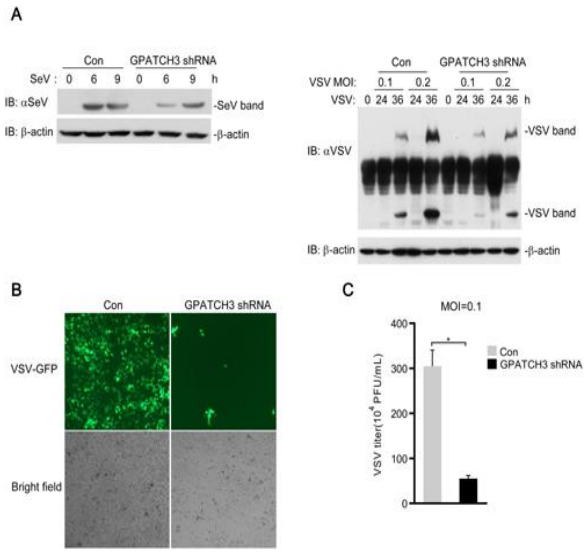
The results have been published in *Journal of Virology* entitled "Quantitative proteomic analysis of mosquito C6/36 cells reveals host proteins involved in Zika virus infection ". The Editor Michael Diamond, a famous virologist evaluated, " This could be an important paper for the journal. “

Link: *http://jvi.asm.org/content/early/2017/04/06/JVI.00554-17.full.pdf+html?sid=d09196e0-c2f2-4a88-b120-c0a065ef3938*

Scientists find a negative regulator of innate antiviral immune responses

Recognition of conserved molecular structures of viruses by the host pattern-recognition receptors (PRRs) initiates innate antiviral immune responses. Several families of PRRs, including Toll-like receptors (TLRs), RIG-like receptors (RLRs), NOD-like receptors (NLRs) and recently identified DNA sensors, have been shown to sense different microbial components.

Previously, the research group led by Prof. WANG Yanyi from Wuhan Institute of Virology of the Chinese Academy of Sciences identified VISA (also named as MAVS, IPS-1 and Cardif) as a critical adaptor of virus-triggered, RLR-mediated induction of innate



antiviral responses. In the present study, the scientists further found that GPATCH3, a functionally uncharacterized protein, interacted with mitochondria-localized VISA upon virus infection and disrupted the assembly of VISA-signalosome.

They reveal that GPATCH3 acts as a negative regulator of VISA and functions as a brake of RLR-mediated antiviral innate responses. Knockdown of GPATCH3 significantly enhanced SeV-triggered induction of downstream antiviral genes in multiple cell lines, including primary PBMCs and HFFs but had no marked effects on TLR3- or DNA sensor-mediated signaling. GPATCH3-deficient cells showed higher induction of IFNB1 compared with wild-type cells upon SeV or VSV infection. Mechanistically, GPATCH3 interacted with VISA and impaired assembly of the VISA-associated signaling complexes. This discovery helps to understand how the innate antiviral responses are delicately regulated.

Link: *http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1006328*

First Synthesis of a Baculovirus Reported

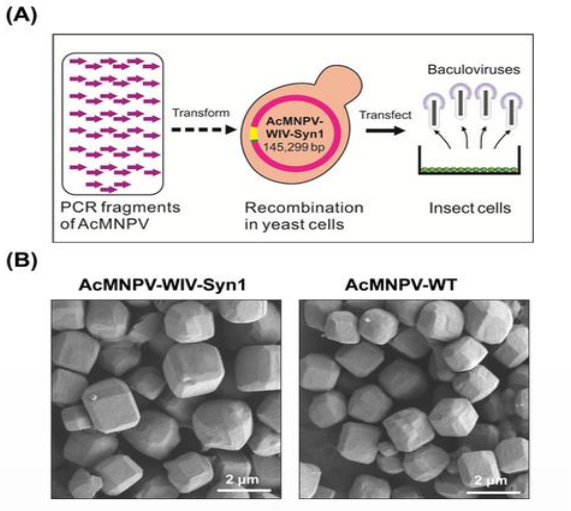
Advances in synthetic biology have come to an age of whole genome synthesis. Synthetic viruses provide a powerful platform to delve deeper into the nature and function of viruses as well as to engineer viruses with novel properties.

To date, most of synthesized viruses have been RNA viruses. RNA viruses normally have a limited genome size with the biggest reported so far are from coronaviruses (~30 kb). In contrast to RNA viruses, only very few DNA viruses have been synthesized, all with a genome size less than 6 kb.

Baculoviruses are insect-specific and are being used rather widely as environmentally benign biocontrol agents against agricultural and forest insect pests. The baculoviral genome is a closed circular superhelical dsDNA of about 80–180 kb.

In a present study, the research group led by Prof. HU Zhihong from Wuhan Institute of Virology of the Chinese Academy of Sciences report that the construction of a synthetic genome of the Autographa californica multiple nucleopolyhedrovirus (AcMNPV), the type species of baculoviruses, by a combination of PCR and transformation-associated recombi-nation (TAR)30 in Saccharomyces cerevisiae.

Infectious progeny virus was obtained after Sf9 insect cells were transfected with the synthetic baculovirus genome. One-step growth curves and other properties showed that the synthetic virus was bio-logically very similar to the native virus.



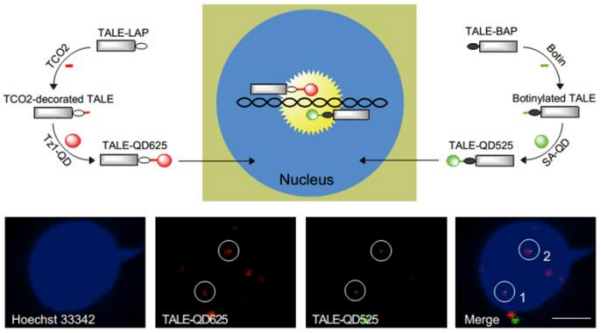
To make sure the fidelity of the synthesized genome, the scientists first sequenced the genome of the parental virus AcMNPV-WT of their virus collection by 454 sequencing.

The AcMNPV-WT genome was found to be nearly identical to AcMNPV-E2 strain31 with only 9 nts differences. Then, a synthetic AcMNPV genome was assembled from the parental sequence by a combination of PCR and TAR in 4 steps.

The new technology provides strong tool for basic study in baculovirus, and can be used to generate the even more powerful baculovirus-based expression vectors. As a significant breakthrough in large DNA virus, this study is considered as "a highly significant paper that will serve as a landmark in synthetic biology" by the peer reviewer.

Link: *http://pubs.acs.org/doi/pdf/10.1021/acssynbio.7b00028*

Scientists map single-copy HIV-1 provirus loci in human chromosomes in live host cells



Single genomic loci are often related to specific cellular functions, genetic diseases, or pathogenic infections. Visualization of single genomic loci in live human cells is currently of great interest, yet it remains challenging.

In a joint study with Prof. ZHANG Xian'en from Institute of Biophysics of the Chinese Academy of Sciences, Prof. CUI Zongqiang from Wuhan Institute of Virology of the Chinese Academy of Sciences has reported a novel approach to allow the visualization of single genomic loci in live cells. This approach combines the sequence-specific recognition of transcription activator-like effectors (TALEs) with the single-particle sensitivity of quantum dots (QDs). Compared with zinc-finger proteins or engineered meganucleases, TALEs are easier to design and optimize for the purpose of sequence-specific binding and labelling.

In this work, they describe a strategy for live cell imaging of single genomic loci by combining transcription activator-like effectors (TALEs) with a quantum dot labelling technique. The scientists design and select a pair of TALEs that specifically target HIV-1 proviral DNA sequences, and use bioorthogonal ligation reactions to label them with different colour quantum dots (QDs). These QD-labelled TALEs are able to enter the cell nucleus to provide fluorescent signals to identify single gene loci.

Based on the co-localization of the pair of different coloured QD-labelled TALEs, the they determine and map single-copy HIV-1 provirus loci in human chromosomes in live host cells.

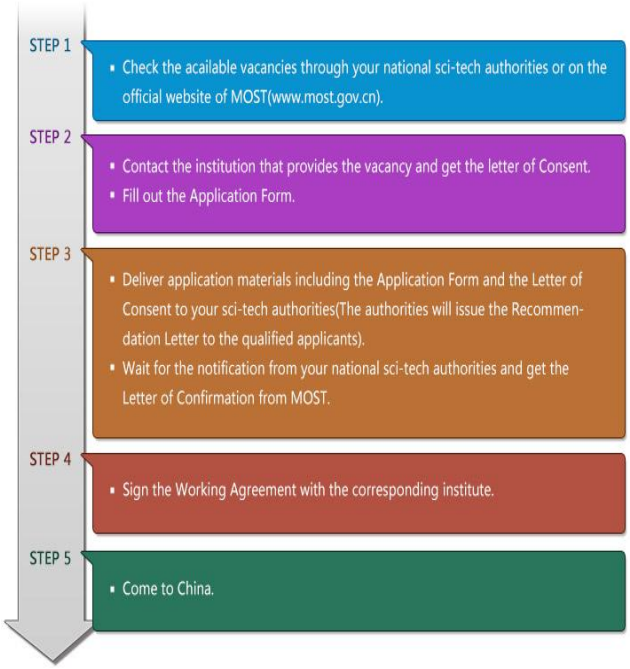
Link: *https://www.nature.com/articles/ncomms15318*

Cooperation

MOST Talented Young Scientist Program

The Talented Young Scientist Program (TYSP) from Mistry of Science and Technology (MOST) supports talented young scientists, scholars and researchers from Afro-Asian countries to work in Chinese research institutes, universities or enterprises. TYSP aims to promote communication among Afro-Asian science and technology talents, nurture young science and technology leaders, and foster long-term international cooperation among research institutes, universities and enterprises in Afro-Asian countries. Ministry of Science and Technology of China (MOST) will provide each scientist with RMB ¥12500 per month for accommodation, insurance and other living expenditure during the program.

For more details: http://www.tysp.org/English



Science Tips

WIV participates in "Science Activity Week" and "Public Science Day"

On May 20th, the 2017 Hubei "Science Activity Week" and CAS "Public Science Day" was launched. WIV presented a science-popularizing feast to the public in the theme of “innovation and development, technology and the future”. WIV not only made the biological safety and 3D printed virus science popularizing exhibition boards, but also brought exquisite 3D printed color virus models, handmade clay models and originally designed beautiful science-popularizing bookmarks. Lots of kids used the clay, watercolor and coloring cards to create the virus world in their mind according to the 3D printed virus models on tables.

Cooperation

Open Lab Day: engaging children with the virus world

On 21 May 2017, Wuhan Institute of Virology held the "Open Lab Day" event. More than 50 children visited to learn the knowledge about virus. The event aims to open doors to the public and engage children with the virus world. The visitors first came to the Virus Specimen Museum of China (VISM). The exquisite 3D printed virus models of Zika virus, hepatitis B virus, adenovirus and others have caught their eyes. Then, an award-winning quiz was held in a lively and humorous form and attracted everybody's active participation. During the last segment of free activities, the kids and their parents positively took part in the "free graffiti", "clay virus model making", "VR experience" and other projects to have an in-depth understanding of the virus world through the hands-on practices and games.

Express News

Upcoming event - The 6th European Virus Archive goes global Annual Conference

The 6th European Virus Archive goes global Annual Conference, jointly organized by Wuhan Institute of Virology, Hubei Society for Microbiology, State Key Laboratory of Virology and *Virologica Sinica* will be held on October 16-18, 2017 in Wuhan, China.

Conference Chairs:
- HU Zhihong (Priciple Investigator, WIV, CAS)
- Jean-Louis Romette (Professor, Aix-Marseille Université)

Conference Sessions:
- International standard of virus resources;
- Global cooperation of virus resources;
- Emergency response of emerging viral diseases;
- Research progress and trend of emerging infectious disease.

端午節

乙巳月 丁巳日
五月初五

Happy Dragon Boat Festival !