Twitter Thread by Billy Bostickson ■■&■ ■

Billy Bostickson ■■&■ ■
@BillyBostickson

Zhengli Shi and Deyin Guo in 2015
Coronavirus nsp10/nsp16 Methyltransferase Can Be Targeted by nsp10-Derived Peptide In Vitro and In Vivo To Increase or Decrease Replication and Pathogenesis
https://t.co/YMlsqFOvZp

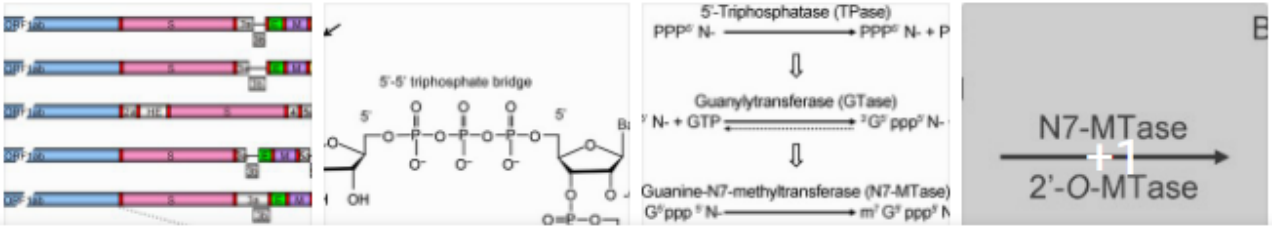
Overview Stats Comments Citations (40) References (49) Related

7:e1002294, 2011, http://dx.doi.org/10.1371/journal.ppat.1002294) . In this study, we demonstrate that stimulation of nsp16 2'-O-MTase activity by nsp10 is a universal and conserved mechanism in coronaviruses, including FCoV, and that nsp10 is functionally interchangeable in the stimulation of nsp16 of different coronaviruses. Based on our current and previous studies, we designed a peptide (TP29) from the sequence of the interaction interface of mouse hepatitis virus (MHV) nsp10 and demonstrated that the peptide inhibits the 2'-O-MTase activity of different coronaviruses in biochemical assays and the viral replication in MHV infection and SARS-CoV replicon models. Interestingly, the peptide TP29 exerted robust inhibitory effects in vivo in MHV-infected mice by impairing MHV virulence and pathogenesis through suppressing virus replication and enhancing type I interferon production at an early stage of infection. Therefore, as a proof of principle, the current results indicate that coronavirus 2'-O-MTase activity can be targeted in vitro and in vivo. Importance: Coronaviruses are important pathogens of animals and human with high zoonotic potential. SARS-CoV encodes the 2'-O-MTase that is composed of the catalytic subunit nsp16 and the stimulatory subunit nsp10 and plays an important role in virus genome replication and evasion from innate immunity. Our current results demonstrate that stimulation of nsp16 2'-O-MTase activity by nsp10 is a common mechanism for coronaviruses, and

Deyin Guo 2016:
Molecular mechanisms of coronavirus RNA capping and methylation
https://t.co/xdEU5goLB6

Abstract and figures

The 5'-cap structures of eukaryotic mRNAs are important for RNA stability, pre-mRNA splicing, mRNA export, and protein translation. Many viruses have evolved mechanisms for generating their own cap structures with methylation at the N7 position of the capped guanine and the ribose 2'-Oposition of the first nucleotide, which help viral RNAs escape recognition by the host innate immune system. The RNA genomes of coronavirus were identified to have 5'-caps in the early 1980s. However, for decades the RNA capping mechanisms of coronaviruses remained unknown. Since 2003, the outbreak of severe acute respiratory syndrome coronavirus has drawn increased attention and stimulated numerous studies on the molecular virology of coronaviruses. Here, we review the current understanding of the mechanisms adopted by coronaviruses to produce the 5'- cap structure and methylation modification of viral genomic RNAs.



Deyin Guo 2012

Short peptides derived from the interaction domain of SARS coronavirus nonstructural protein nsp10 can suppress the 2′-O-methyltransferase activity of nsp10/nsp16 complex

https://t.co/x30jPYFDTa

Abstract

Coronaviruses are the etiological agents of respiratory and enteric diseases in humans and livestock, exemplified by the life-threatening severe acute respiratory syndrome (SARS) caused by SARS coronavirus (SARS-CoV). However, effective means for combating coronaviruses are still lacking. The interaction between nonstructural protein (nsp) 10 and nsp16 has been demonstrated and the crystal structure of SARS-CoV nsp16/10 complex has been revealed. As nsp10 acts as an essential trigger to activate the 2'-O-methyltransferase activity of nsp16, short peptides derived from nsp10 may have inhibitory effect on viral 2'-O-methyltransferase activity. In this study, we revealed that the domain of aa 65-107 of nsp10 was sufficient for its interaction with nsp16 and the region of aa 42-120 in nsp10, which is larger than the interaction domain, was needed for stimulating the nsp16 2'-O-methyltransferase activity. We further showed that two short peptides derived from the interaction domain of nsp10 could inhibit the 2'-O-methyltransferase activity of SARS-CoV nsp16/10 complex, thus providing a novel strategy and proof-of-principle study for developing peptide inhibitors against SARS-CoV.

Deyin Guo 2013

Structure-function Analysis of SARS Coronavirus RNA Cap Guanine-N7 Methyltransferase.

https://t.co/NG57b85hcs

Coronaviruses possess a cap structure at the 5' -end of viral genomic RNA and subgenomic RNAs, which is generated through consecutive methylations by virally encoded (guanine-N7-)-methyltransferase (N7-MTase) and 2' -O-methyltransferase (2' -O-MTase). The coronaviral N7-MTase is unique for its physical linkage with an exoribonuclease (ExoN) harbored in the nonstructural protein (nsp) 14 of coronaviruses. In this study, the structure-function relationships of the N7-MTase were analyzed by deletion and site-directed mutagenesis of SARS coronavirus (SARS-CoV) nsp14. The results showed that the ExoN domain is closely involved in the activity of the N7-MTase, suggesting that coronavirus N7-MTase is different from all other viral N7-MTases, which are separable from other structural domains located in the same polypeptide. Two of the 12 critical residues, identified to be essential for the N7-MTase, were located at the N-terminus of the core ExoN domain, reinforcing a role of the ExoN domain in the N7-MTase activity of nsp14. The other 10 critical residues were distributed throughout the N7-MTase domain but mainly localized in the S-adenosyl-L-methionine (SAM)-binding pocket and key structural elements of the MTase fold of nsp14. The sequence motif DxGxPxA (aa 331-338) was identified as the key part of the SAM-binding site. These results provide insights into the structure and functional mechanisms of coronaviral nsp14 N7-MTase.

Deyin Guo 2016

Identification and Characterization of a Ribose 2'-O-Methyltransferase Encoded by the Ronivirus Branch of Nidovirales

https://t.co/zZ5V7erseD

Abstract and figures

Importance: Methylation of the 5' -cap structure of viral RNAs plays important roles in genome replication and evasion of innate recognition of viral RNAs by cellular sensors. It is known that coronavirus nsp14 acts as an N7-(guanine)-methyltransferase (MTase) and nsp16 as 2' -O-MTase, which are involved in the modification of RNA cap structure. However, these enzymatic activities have not been shown for any other nidoviruses beyond coronaviruses in the order Nidovirales. In this study, we identified a 2' -O-methyltransferase encoded by ronivirus and it shows common and unique features in comparison with that of coronaviruses. Ronivirus 2' -O-MTase does not need a protein co-factor for MTase activity whereas coronavirus nsp16 needs the stimulating factor nsp10 for its full activity. The conserved K-D-K-E catalytic tetrad is identified in ronivirus 2' -O-MTase. These results extend our understanding of nidovirus RNA capping and methylation beyond coronaviruses and also strengthen the evolutionary and functional link between roniviruses and coronaviruses.

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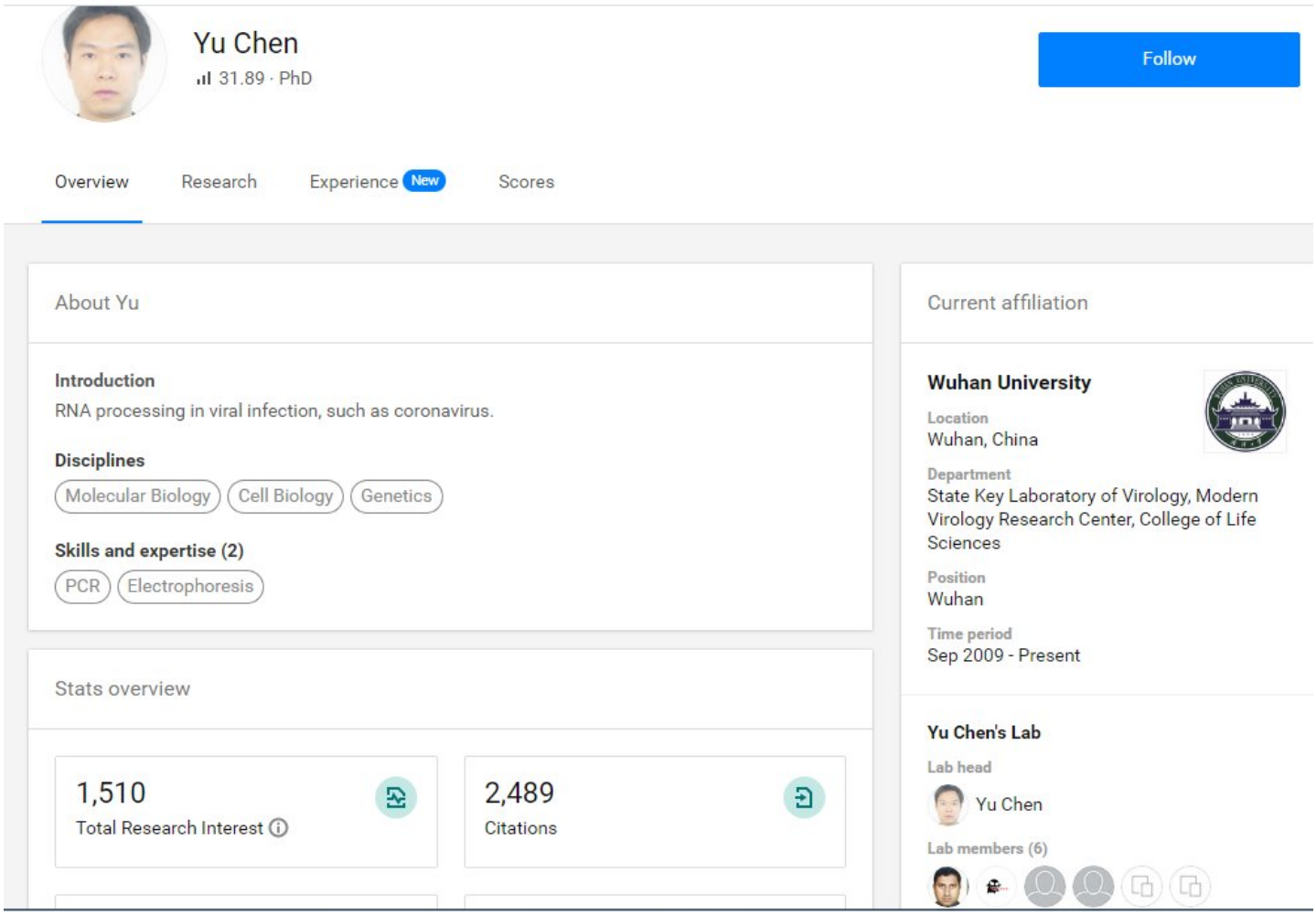


Deyin Guo 2009
Functional screen reveals SARS coronavirus nonstructural protein nsp14 as a novel cap N7 methyltransferase
https://t.co/9xN1Y8cSV5

Abstract and figures

The N7-methylguanosine (m7G) cap is the defining structural feature of eukaryotic mRNAs. Most eukaryotic viruses that replicate in the cytoplasm, including coronaviruses, have evolved strategies to cap their RNAs. In this report, we used a yeast genetic system to functionally screen for the cap-forming enzymes encoded by severe acute respiratory syndrome (SARS) coronavirus and identified the nonstructural protein (nsp) 14 of SARS coronavirus as a (guanine-N7)-methyltransferase (N7-MTase) in vivo in yeast cells and in vitro using purified enzymes and RNA substrates. Interestingly, coronavirus nsp14 was previously characterized as a 3'-to-5' exoribonuclease, and by mutational analysis, we mapped the N7-MTase domain to the carboxy-terminal part of nsp14 that shows features conserved with cellular N7-MTase in structure-based sequence alignment. The exoribonuclease active site was dispensable but the exoribonuclease domain was required for N7-MTase activity. Such combination of the 2 functional domains in coronavirus nsp14 suggests that it may represent a novel form of RNA-processing enzymes. Mutational analysis in a replicon system showed that the N7-MTase activity was important for SARS virus replication/transcription and can thus be used as an attractive drug target to develop antivirals for control of coronaviruses including the deadly SARS virus. Furthermore, the observation that the N7-MTase of RNA life could function in lieu of that in DNA life provides interesting evolutionary insight and practical possibilities in antiviral drug screening.

Deyin Guo 2011

Biochemical and Structural Insights into the Mechanisms of SARS Coronavirus RNA Ribose 2′-O-Methylation by nsp16/nsp10 Protein Complex

https://t.co/N2iMtjkZ0c

Figure 7. (Lower Right) Structural mechanisms of nsp10 in stimulating the binding of capped RNA to nsp16.

The 5'-cap structure is a distinct feature of eukaryotic mRNAs, and eukaryotic viruses generally modify the 5'-end of viral RNAs to mimic cellular mRNA structure, which is important for RNA stability, protein translation and viral immune escape. SARS coronavirus (SARS-CoV) encodes two S-adenosyl-L-methionine (SAM)-dependent methyltransferases (MTase) which sequentially methylate the RNA cap at guanosine-N7 and ribose 2'-O positions, catalyzed by nsp14 N7-MTase and nsp16 2'-O-MTase, respectively. A unique feature for SARS-CoV is that nsp16 requires non-structural protein nsp10 as a stimulatory factor to execute its MTase activity. Here we report the biochemical characterization of SARS-CoV 2'-O-MTase and the crystal structure of nsp16/nsp10 complex bound with methyl donor SAM. We found that SARS-CoV nsp16 MTase methylated m7GpppA-RNA but not m7GpppG-RNA, which is in contrast with nsp14 MTase that functions in a sequence-independent manner. We demonstrated that nsp10 is required for nsp16 to bind both m7GpppA-RNA substrate and SAM cofactor. Structural analysis revealed that nsp16 possesses the canonical scaffold of MTase and associates with nsp10 at 1:1 ratio. The structure of the nsp16/nsp10 interaction interface shows that nsp10 may stabilize the SAM-binding pocket and extend the substrate RNA-binding groove of nsp16, consistent with the findings in biochemical assays. These results suggest that nsp16/nsp10 interface may represent a better drug target than the viral MTase active site for developing highly specific anti-coronavirus drugs.

Deyin Guo's 2017 "Archived" HIV Project...or was it?
Cellular factors regulating HIV-1 replication
https://t.co/SOieGZtvLI

Research referenced in this project

Regulation of the Alternative Splicing and Function of Cyclin T1 by the Serine-Arginine-Rich Protein ASF/SF2

Article Full-text · Apr 2017 · Journal of Cellular Biochemistry

Jieqiong Zhou · Guozhen Gao · Panpan Hou · [...] · Deyin Guo

View 1 Citation

M2 pyruvate kinase enhances HIV-1 transcription from its long terminal repeat

Article Full-text · Apr 2014 · Frontiers of Biology in China

Xiaoyun wu · Guozhen Gao · Musarat Ishaq · [...] · Deyin Guo

View

Inhibition of HIV-1 Transcription and Replication by a Newly Identified Cyclin T1 Splice Variant

Article Full-text · Apr 2013 · Journal of Biological Chemistry

Guozhen Gao · Xiaoyun wu · Jieqiong Zhou · [...] · Deyin Guo

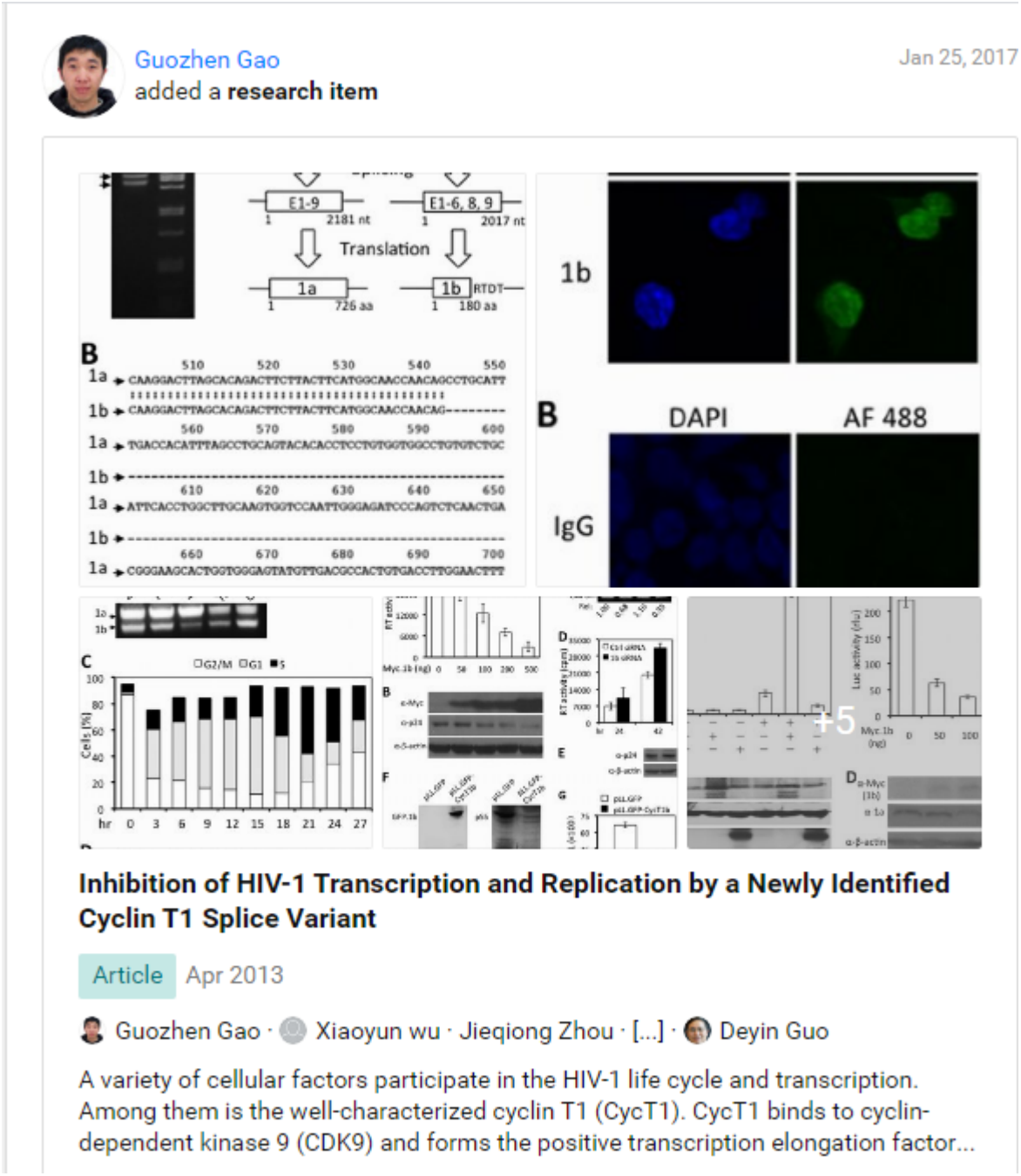
View 7 Citations

Deyin Guo and Researchers from the State Key Laboratory of Virology, College of Life Sciences, Wuhan University, 2015
The nucleocapsid protein of coronaviruses acts as a viral suppressor of RNA silencing in mammalian cells
https://t.co/mp5MJH9Aho

Abstract

© 2015, American Society for Microbiology. RNA interference (RNAi) is a process of eukaryotic posttranscriptional gene silencing that functions in antiviral immunity in plants, nematodes, and insects. However, recent studies provided strong supports that RNAi also plays a role in antiviral mechanism in mammalian cells. To combat RNAi-mediated antiviral responses, many viruses encode viral suppressors of RNA silencing (VSR) to facilitate their replication. VSRs have been widely studied for plant and insect viruses, but only a few have been defined for mammalian viruses currently. We identified a novel VSR from coronaviruses, a group of medically important mammalian viruses including Severe acute respiratory syndrome coronavirus (SARS-CoV), and showed that the nucleocapsid protein (N protein) of coronaviruses suppresses RNAi triggered by either short hairpin RNAs or small interfering RNAs in mammalian cells. Mouse hepatitis virus (MHV) is closely related to SARS-CoV in the family Coronaviridae and was used as a coronavirus replication model. The replication of MHV increased when the N proteins were expressed in trans, while knockdown of Dicer1 or Ago2 transcripts facilitated the MHV replication in mammalian cells. These results support the hypothesis that RNAi is a part of the antiviral immunity responses in mammalian cells.

Back to Deyin Guo's "archived" HIV Project
1. Paper 1:
Inhibition of HIV-1 Transcription and Replication by a Newly Identified Cyclin T1 Splice Variant (April 2013)



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2. Paper 2:

Regulation of the Alternative Splicing and Function of Cyclin T1 by the Serine-Arginine-Rich Protein ASF/SF2

https://t.co/7dEiHnJ1jb

Abstract and figures

Positive transcription elongation factor-b (P-TEFb) is required for the release of RNA polymerase II (RNAPII) from its pause near the gene promoters and thus for efficient proceeding to the transcription elongation. It consists of two core subunits – CDK9 and one of T-typed or K-typed cyclin, of which, cyclin T1/CDK9 is the major and most studied combination. We have previously identified a novel splice variant of cyclin T1, cyclin T1b, which negatively regulates the transcription elongation of HIV-1 genes as well as several host genes. In this study, we revealed the serine-arginine-rich protein, ASF/SF2, as a regulatory factor of the alternative splicing of cyclin T1 gene. ASF/SF2 promotes the production of cyclin T1b versus cyclin T1a and regulates the expression of cyclin T1-depedent genes at the transcription level. We further found that a cis-element on exon 8 is responsible for the skipping of exon 7 mediated by ASF/SF2. Collectively, ASF/SF2 is identified as a splicing regulator of cyclin T1, which contributes to the control of the subsequent transcription events.

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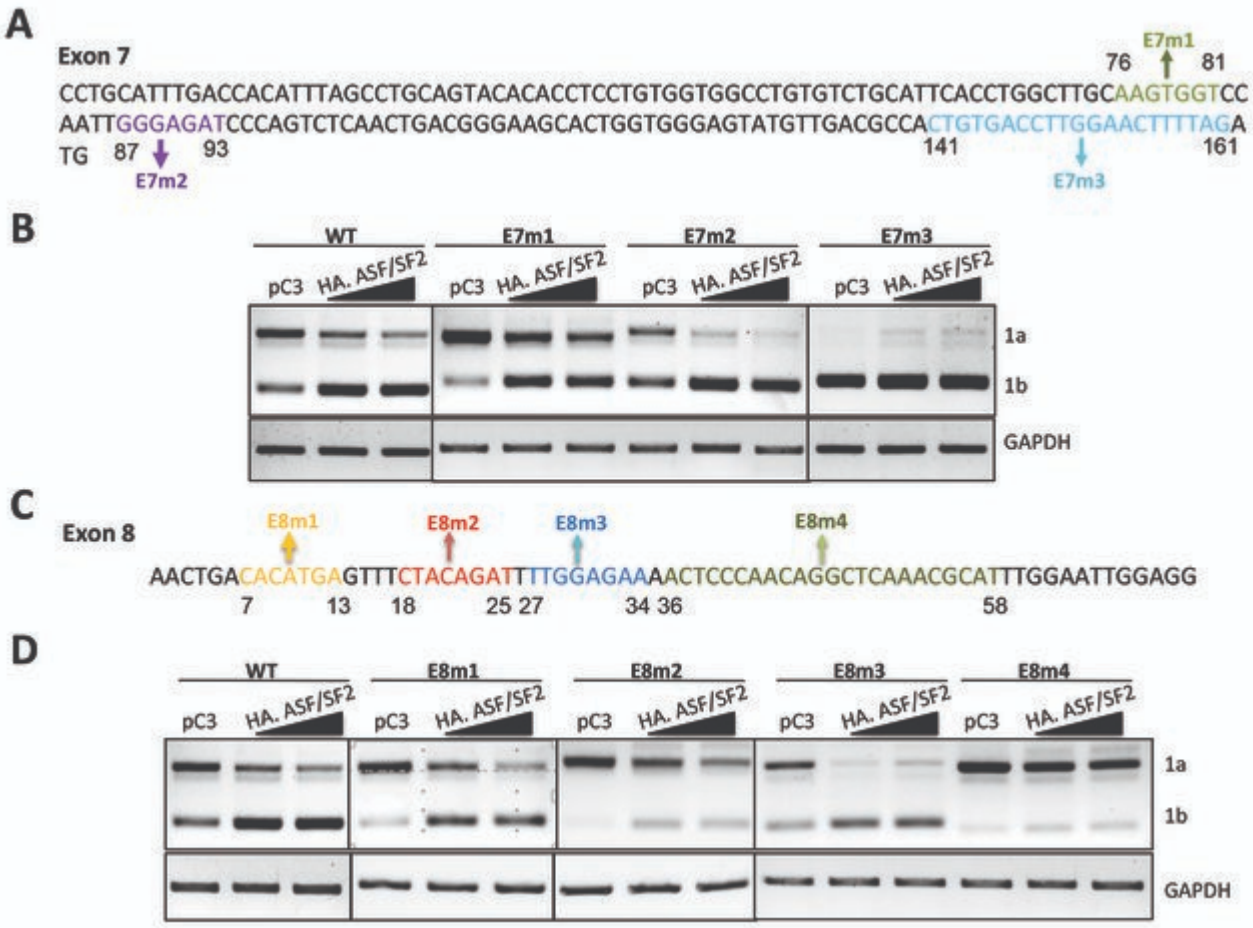
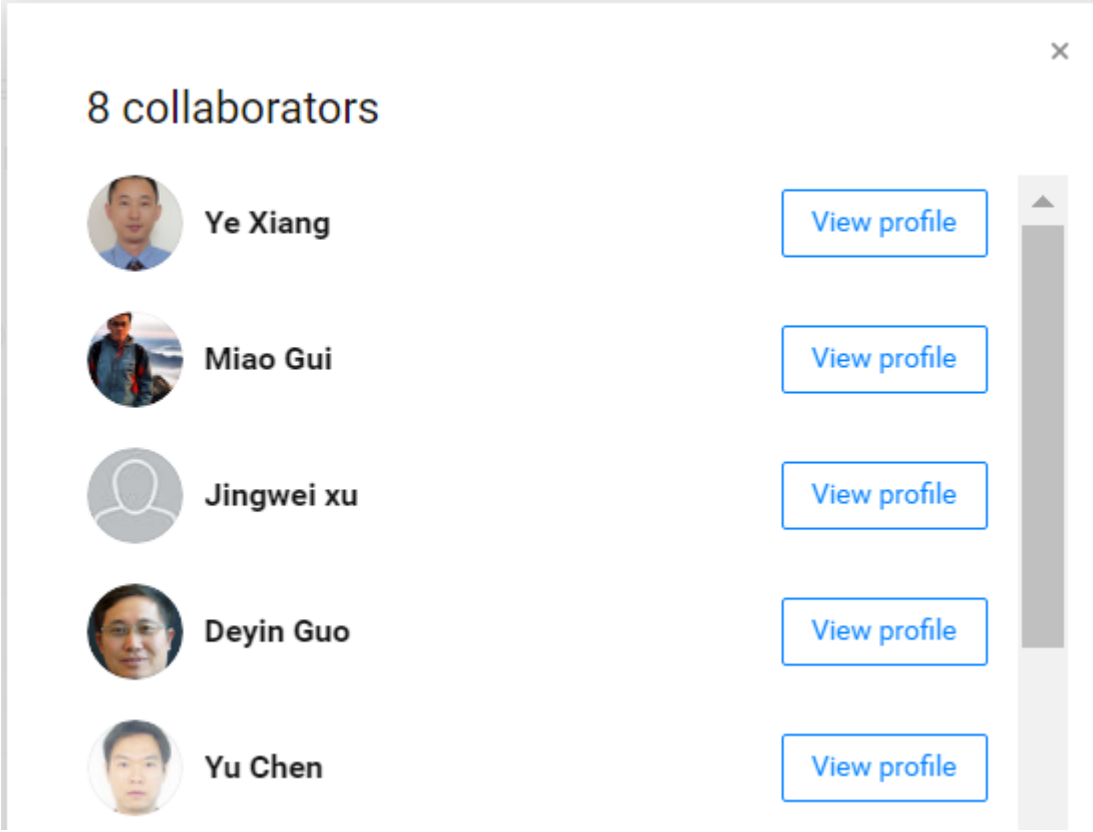
3. Paper 3:

M2 pyruvate kinase enhances HIV-1 transcription from its long terminal repeat

https://t.co/GfnHDPOTAR



From Coronavirus to HIV and back to Coronavirus with Deyin Guo and friends:
https://t.co/a2kjcwVBkM
https://t.co/zVzx0CgLTm



Deyin Guo's Tsinghua University Project Associates 2018
Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2
https://t.co/zVzx0CgLTm

The trimeric SARS coronavirus (SARS-CoV) surface spike (S) glycoprotein consisting of three S1-S2 heterodimers binds the cellular receptor angiotensin-converting enzyme 2 (ACE2) and mediates fusion of the viral and cellular membranes through a pre- to postfusion conformation transition. Here, we report the structure of the SARS-CoV S glycoprotein in complex with its host cell receptor ACE2 revealed by cryo-electron microscopy (cryo-EM). The complex structure shows that only one receptor-binding domain of the trimeric S glycoprotein binds ACE2 and adopts a protruding “up” conformation. In addition, we studied the structures of the SARS-CoV S glycoprotein and its complexes with ACE2 in different in vitro conditions, which may mimic different conformational states of the S glycoprotein during virus entry. Disassociation of the S1-ACE2 complex from some of the prefusion spikes was observed and characterized. We also characterized the rosette-like structures of the clustered SARS-CoV S2 trimers in the postfusion state observed on electron micrographs. Structural comparisons suggested that the SARS-CoV S glycoprotein retains a prefusion architecture after trypsin cleavage into the S1 and S2 subunits and acidic pH treatment. However, binding to the receptor opens up the receptor-binding domain of S1, which could promote the release of the S1-ACE2 complex and S1 monomers from the prefusion spike and trigger the pre- to postfusion conformational transition.

More from the Coronavirus Project
https://t.co/16HCDJg7Vc
2017 Deyin Guo
Electron microscopy studies of the coronavirus ribonucleoprotein complex
https://t.co/etEBKfeunu
Coronaviruses: genome structure, replication, and pathogenesis (Deyin Guo 2020)
https://t.co/LANkA4X3WJ

Protein Cell
DOI 10.1007/s13238-016-0352-8

Protein & Cell

LETTER

Electron microscopy studies of the coronavirus ribonucleoprotein complex

Dear Editor,

Coronaviruses are enveloped viruses that cause different diseases in humans and animals (Su et al., 2016). Murine hepatitis virus (MHV) causes hepatitis, enteritis and central nervous system diseases in rodents and is one of the best-studied coronaviruses. MHV belongs to the genera betacoronavirus. Members from the same genera also include highly pathogenic coronaviruses such as the severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle-East respiratory syndrome coronavirus (MERS-CoV) (Vijay and Perlman, 2016).

The coronavirus has a single strand, positive sense RNA genome of about 30 kb, which encodes 4–5 structural proteins, including the nucleocapsid (N) protein, the matrix (M) protein, the small envelope (E) protein, the spike (S) glycoprotein and for some betacoronaviruses, the hemagglutinin esterase (HE) protein (Su et al., 2016). The N proteins bind the viral RNA genome and play important roles in packaging and stabilizing the virus genome, in viral particle assembly and envelope formation, and in the genomic RNA synthesis (McBride et al., 2014). Moreover, it was reported that coronavirus nucleoprotein can regulate host cell cycle, cell stress response, and influence the immune system and other cellular responses (Lu et al., 2011; McBride et al., 2014; Cui et al., 2015; Chang et al., 2016). The N proteins of different coronaviruses are homologous and can be divided into five parts and domains: the N terminal flexible arm, the N terminal domain (NTD), the middle disordered region (LKR), the C terminal domain (CTD), the C terminal flexible tail. The N terminal arm, C terminal tail and the LKR are flexible (Chang et al., 2014). The NTD structures of MHV, SARS-CoV, infectious bronchitis virus (IBV), human coronavirus strain OC43 (HCoV OC43) and the CTD structures of MHV, SARS-CoV and IBV were determined using either x-ray crystallography or NMR (Chang et al., 2016). The determined NTD or CTD structures are highly similar among different coronaviruses. Both the NTD and CTD are shown to interact with the genome RNA while the CTD is also responsible for the dimerization of the nucleoproteins (Chang et al., 2014). The domain crystal structures have provided useful information on the assembly of the ribonucleoprotein complex (RNP), but a lack of the full-length N protein structure and the RNP structure limits our understanding to the assembly and function of coronavirus RNP.

Previous analysis of the RNP extracted from the virus by using negative staining electron microscopy showed that coronavirus RNP might be a long helix with a diameter between 9 nm to 16 nm (Macneughton and Davies, 1978). In this study, we isolated the RNPs from MHV and performed negative staining EM and cryo-EM images analysis of the isolated intact and degraded RNPs. We found that the isolated RNPs are in either relaxed helical sausage-like or supercoiled flower-like structures. Interestingly, we also found that the isolated intact RNPs degraded into small pothook-like subunits. These small subunits could be the building blocks of the long loose helical and the supercoiled flower-like RNP structures.

We performed both negative staining EM and cryo-EM analysis of the MHV (strain MHV-A59) particles. Negative staining images of the intact MHV particles showed that most viral particles had a round shape while some distorted particles were also observed (Fig. 1A). Cryo-EM image analysis of the same sample showed almost all round shaped particles (Fig. 1B), indicating that the distortion in the negative staining images might be caused by the staining procedure. The corona-like spikes around the envelope could be identified in both the negative staining images (Fig. 1A) and cryo-EM images (Fig. 1B). The cryo-EM MHV particles were picked and subjected for 2D classification analyzes. The results showed that the particles have a diameter of ~80 nm to 90 nm (Fig. 1C), which is consistent with the previous EM results (Neuman et al., 2006; Barcena et al., 2009). A dense interior core corresponding to the intertwined RNP is encapsulated inside the envelope (Fig. 1C).

We then broke the MHV particles by incubating the particles in a lysis buffer containing ~3% CHAPS. Negative staining analysis showed that most of the virus particles were broken and the RNPs were released after the treatment. The released RNPs are in either a loose filament structure or in a compact flower-like assembly that may be similar to the intact RNP assembly in virus particles (Fig. 1D). There were also some smaller particles, which might be the RNP fragments (Fig. 1D).

SDS-PAGE gel analysis of the intact virus showed that the N protein is about 55 kDa (Fig. 1E). To investigate the

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Deyin Guo and his Lab (October 2019)

P200 family protein IFI204 negatively regulates type I interferon responses by targeting IRF7 in nucleus

https://t.co/f0Tyzfqjx8

P200 family protein IFI204 negatively regulates type I interferon responses by targeting IRF7 in nucleus

October 2019 · PLoS Pathogens 15(10):e1008079 · Follow journal

DOI: 10.1371/journal.ppat.1008079

Lab: Deyin Guo's Lab

Liu Cao · Yanxi Ji · Lanyi Zeng · Show all 12 authors · Deyin Guo

This research has to be considered in the light of other research by Deyin Guo (& allegations)
See here:
Serial passage in Wuhan with Deyin Guo!
https://t.co/r3qTaJdivw
CCR5 editing by SA Cas9 in human primary CD4+ T cells promotes HIV-1 resistance
https://t.co/PA26YQ7qIx

> 11/ Serial passage in Wuhan with Deyin Guo!
> To understand adaptation of H5N1 to mammals, a non-pathogenic H5N1 virus (HN021) in mice was passaged 15 times in mammals.
> Experiment showed mouse-adapted variants highly pathogenic in mice after passageshttps://t.co/QBLpvNVg0g
>
> — Billy Bostickson \U0001f3f4\U0001f441&\U0001f441 \U0001f193 (@BillyBostickson) June 20, 2020

Tweets 35 - 55 on Deyin Guo here:
https://t.co/2WctrvmLp3

> 35/ x More Experiments
> By the way, I just noticed that experiment in last tweet took place in May/June 2019 & co-author was Deyin Guo, accused by Miles Guo of being the scientists who designed SARS-COV-2, who is now at Sun Yat Sen University but still experiments at WHU ABSL-3 pic.twitter.com/5CKNXAGDSm
>
> — Billy Bostickson \U0001f3f4\U0001f441&\U0001f441 \U0001f193 (@BillyBostickson) June 3, 2020

The End?
Unroll @threadreaderapp


武漢肺炎