新型冠状病毒信息简报

第 8 期（2020 年 3 月 26 日报）

上海科技大学免疫化学研究所

生物医学大数据平台和高通量筛选平台联合编译制作

联系人：蒋立春 jianglch@shanghaitech.edu.cn

内容介绍

分类	标题名称
疫情播报	1. 2020 年 3 月 25 日疫情(SARS-CoV-2 pandemic)
观点评述	2. COVID-19 需要一个曼哈顿计划(COVID-19 needs a Manhattan Project) 3. COVID-19 感染：基于免疫反应的观点 （COVID-19 infection: the perspectives on immune responses)
基础研究	4. 替洛隆：一种广谱的抗新发病毒药物 （Tilorone: A Broad-Spectrum Antiviral For Emerging Viruses） 5. 利用 RNA 直接测序和串联质谱技术对 SARS-CoV-2 的转录组和蛋白质组进行研究，发现细胞传代可以导致 SARS-CoV-2 的刺突蛋白（S 蛋白）基因发生框内缺失突变，进而导致刺突蛋白丢失类弗林蛋白酶切割位点 （Characterisation of the transcriptome and proteome of SARS-CoV-2 using direct RNA sequencing and tandem mass spectrometry reveals evidence for a cell passage induced in-frame deletion in the spike glycoprotein that removes the furin-like cleavage site） 6. 冠状病毒的核糖核酸通过切割病毒的 polyU 序列进而帮助病毒逃避宿主的免疫监测（冠状病毒的核糖核酸通过切割病毒的 polyU 序列进而帮助病毒逃避宿主的免疫监测 （Coronavirus endoribonuclease targets viral polyuridine sequences to evade activating host sensors） 7. 通过示踪 RNA 的合成揭示关于冠状病毒复制的统一结构和功能模型 （A unifying structural and functional model of the coronavirus replication organelle: tracking down RNA synthesis）

1. 2020 年 3 月 25 日疫情

数据来源：WHO
发布时间：2020 年 3 月 25 日北京时间下午 5 点
链接：https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports
根据 WHO 提供的数据，2020 年 3 月 25 日全球累计确诊新型冠状病毒病人 414179 例，当日新增确诊 40712 例，累计死亡 18440 例，当日新增死亡 2202。
中国累计确诊 81848 例，累计死亡 3287 例，当日新增确诊 101 例，新增死亡 4 例。

2. COVID-19 需要一个曼哈顿计划

COVID-19 needs a Manhattan Project
来源： Science
发布时间：2020-03-25
来源链接： https://science.sciencemag.org/content/early/2020/03/25/science.abb8654
内容摘要：

全球疫苗免疫联盟(Gavi)首席执行官 Seth Berkley 于 2020 年 3 月 25 日在《Science》发文称，至少有 44 种疫苗处于早期开发阶段，但是，目前的零星努力是不够的。COVID-19 需要一个曼哈顿计划，汇集全球的专业知识和资源，采取大规模、协调一致的方法来开发一种 SARS-CoV-2 疫苗。不仅可能拯救数十万人的生命，而且还将帮助世界更好地为下一次大流行做好准备。

如此大规模的行动并非易事。信息和资源的特别共享将是至关重要的，包括关于病毒、各种候选疫苗、疫苗佐剂、细胞系和生产进展的数据。所有试验需要并行进行，而不是顺序进行，包括合适的实验设计、速度优化和在不同人群中进行试验——发达国家和发展中国家，从儿童到老年人——这样我们才能最终保护所有人。所有这些也是需要大量资金的，需要一个具有最高质量科学咨询机制的小组领导，虽然这一切都很难实现，但是需要这样做。在许多方面，COVID-19 比其他大科学项目更像曼哈顿计划，不仅因为它涉及到科学的应用，而且不仅仅是在规模上，还因为它是一个全球安全问题。

There is an unprecedented race to develop a vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). With at least 44 vaccines in early-stage development, what outcome can we expect? If we want to maximize the chances for success and have enough doses to end the coronavirus disease 2019 (COVID-19) pandemic, current piecemeal efforts won't be enough. Taking this big, coordinated approach to developing a SARS-CoV-2 vaccine will not only potentially save hundreds of thousands of lives, but will also help the world be better prepared for the next pandemic. An initiative of this scale won't be easy. Extraordinary sharing of information and resources will be critical, including data on the virus, the various vaccine candidates, vaccine adjuvants, cell lines, and manufacturing advances. Trials need to be carried out in parallel, not sequentially, using adaptive trial designs, optimized for speed and tested in different populations—rich and developing countries, from children to the elderly—so that we can ultimately protect everyone. In many ways, COVID-19 is more like the Manhattan Project than other big science efforts, not just because it involves the application of science and not just in terms of scale, but because it is a global security issue.

3. COVID-19 感染：基于免疫反应的观点

COVID-19 infection: the perspectives on immune responses

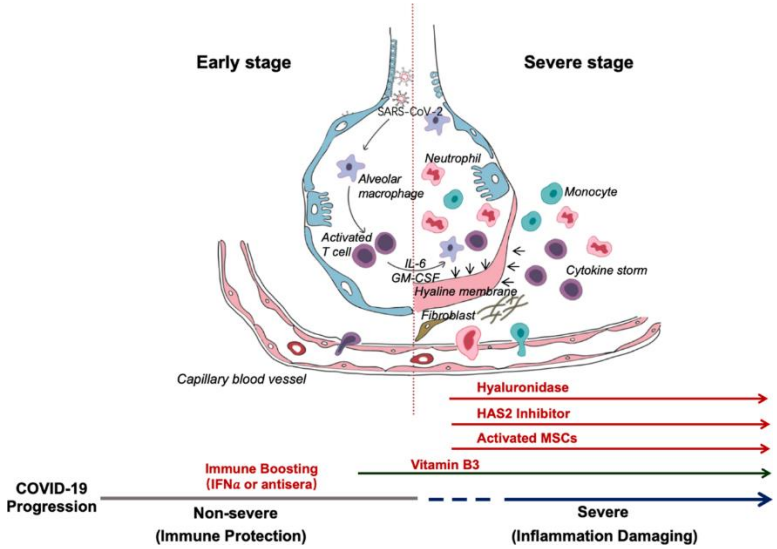
来源：Cell Death & Differentiation

发布时间：2020-3-26

论文链接：https://www.nature.com/articles/s41418-020-0530-3

本文主要是基于某些临床常识，提出了一些简单但基本上被忽略的 COVID-19 患者的治疗方法。作者认为将 COVID-19 疾病进程划分为两阶段非常重要：第一个是基于免疫防御的保护阶段和第二个是炎症驱动的破坏阶段。医生应在第一阶段使用抗血清、干扰素 α （IFN α）等尝试增强免疫反应，而在第二阶段使用 IL-6 抗体、IFN γ 预处理的间充质干细胞（MSC）等来抑制免疫反应。由于维生素 B3 具有高度的肺保护作用，因此应在咳嗽开始后立即使用。当呼吸困难变得明显时，可以在气管内使用透明质酸酶，同时可以使用 4-MU，一种透明质酸合成酶抑制剂，来抑制透明质酸合成。此外，HLA 分型的研究将为规划预防、治疗、疫苗接种和临床方案提供易感性方面的信息。

Overall, this synopsis is based on some clinical common sense. We propose some simple, but largely ignored, approaches to the treatment of COVID-19 patients (Fig. 1). We believe that the two-phase division is very important: the first immune defense-based protective phase and the second inflammation-driven damaging phase. Doctors should try to boost immune responses during the first, while suppressing it in the second phase. Since Vitamin B3 is highly lung protective, it should be used as soon as coughing begins. When breathing difficulty becomes apparent, hyaluronidase can be used intratracheally and at the same time 4-MU can be given to inhibit HAS2. Of course, HLA typing will provide susceptibility information for strategizing prevention, treatment, vaccination, and clinical approaches. We hope that some of the above ideas can be employed to help combat this deadly contagious disease of increasing incidence around the world.



4. 替洛隆：一种广谱的抗新发病毒药物

Tilorone: A Broad-Spectrum Antiviral For Emerging Viruses

来源： Antimicrobial Agents and Chemotherapy, AAC

发布时间：2020-03-23

来源链接： https://aac.asm.org/content/aac/early/2020/03/18/AAC.00440-20.full.pdf
内容摘要：

替洛隆（Tilorone）是一种有 50 年历史的具有抗病毒活性的合成小分子化合物，被认为可以在口服后诱导干扰素的产生。这种药物在俄罗斯联邦的多个国家被用作广谱抗病毒药物。来自美国合作制药公司的 Sean Ekins 和斯坦福国际研究院的 Peter B. Madrid，他们已经描述过了替洛隆（Tilorone）对抗埃博拉病毒的体内和体外活性。在广泛筛选其他病毒后，他们现在展示了针对基孔肯雅病毒(CHIK)和中东呼吸综合征冠状病毒(MERS-CoV)的体外活性。研究结果表明，替洛隆（Tilorone）在体外对 CHIK 和 MERS 有活性。最近爆发的病毒（如 SARS-CoV-2）迫切需要重新评估这种化合物作为广谱抗病毒药物的作用，因为我们尚未充分认识到这种 50 年前发现的药物的效用。

Tilorone is a 50-year-old synthetic small-molecule compound with antiviral activity that is proposed to induce interferon after oral administration. This drug is used as a broad spectrum antiviral in several countries of the Russian Federation. We have recently described activity in vitro and in vivo against the Ebola Virus. After a broad screening of additional viruses, we now describe in vitro activity against Chikungunya virus (CHIK) and Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV). Tilorone has in vitro activity against CHIK and MERS. Recent virus outbreaks such as SARS-CoV-2 suggest the urgent need for reassessment of this compound as a broad-spectrum antiviral as we have yet to fully appreciate the utility of this drug discovered 50 years ago.

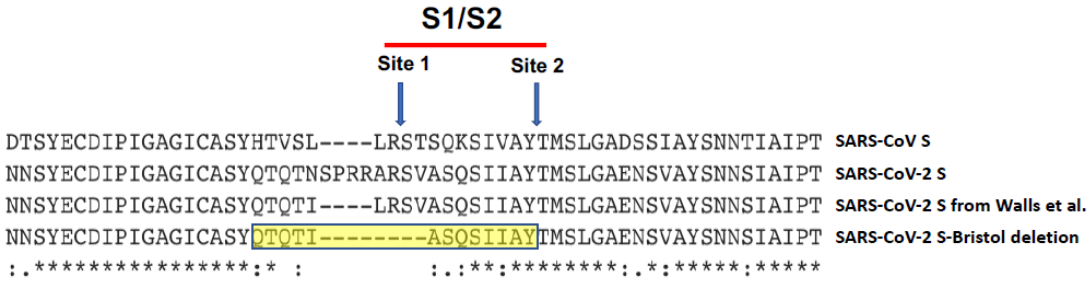
5. 利用 RNA 直接测序和串联质谱技术对 SARS-CoV-2 的转录组和蛋白质组进行研究，发现细胞传代可以导致 SARS-CoV-2 的刺突蛋白（S 蛋白）基因发生框内缺失突变，进而导致刺突蛋白丢失类弗林蛋白酶切割位点

Characterisation of the transcriptome and proteome of SARS-CoV-2 using direct RNA sequencing and tandem mass spectrometry reveals evidence for a cell passage induced in-frame deletion in the spike glycoprotein that removes the furin-like cleavage site

来源：biorxiv
发布时间：2020-03-24
来源链接：https://www.biorxiv.org/content/10.1101/2020.03.22.002204v1
内容摘要：

来自英国布里斯托大学，英国公共卫生部等单位的研究人员利用 MinION 牛津纳米孔测序仪对经 Vero E6 细胞系培养的 SARS-CoV-2 转录组直接进行 RNA 测序，SARS-CoV-2 病毒株为 England/2/2020 (VE6-T)。Vero E6 细胞系已被广泛用于培养新型冠状病毒。研究人员利用最近开发的以 ORF 为中心的分析流程来分析病毒转录组。分析结果显示病毒转录的模式（例如，亚基因组 mRNAs），符合冠状病毒的复制和转录预测模式。在编码刺突蛋白的亚基因组 mRNAs 中检测到 24nt 的读码框内的缺失。该缺失出现在一半以上的比对成功的转录本中，该研究预测这种缺失会导致刺突蛋白失去原来的类弗林蛋白酶切割位点（图一）。该特征序列（motif）在病毒进入或离开宿主细胞时将刺突蛋白切割成功能亚单位。刺突蛋白的裂解可能对人畜共患的冠状病毒传播和致病性造成影响。与转录组分析相结合，串联质谱技术鉴定出了 500 多个病毒肽和 44 个磷酸肽，涵盖了几乎所有计算预测的 SARS-CoV-2 基因组编码的蛋白质，也包含了刺突蛋白缺失变体特有的多肽。在刺突蛋白的类弗林蛋白酶切割位点检测到明显的缺失，进一步证实了 SARS-CoV-2 蛋白的这一区域和其他区域可能容易发

生突变。鉴于刺突蛋白是一种潜在的疫苗靶点，另外，类弗林蛋白酶切割位点可能在该病毒的发病机理和人畜共患病中起到重要作用，因此该发现有重要的意义。在科学研究，动物模型构建和临床样本中涉及到病毒原代的培养，应仔细监测病毒基因组序列。这种变异可能会导致不同的毒力、发病率和死亡率。



图一 刺突蛋白中的缺失片段

文中出现的重要文献（按原文中编号）：

8. Kim, D. et al. The architecture of SARS-CoV-2 transcriptome. bioRxiv, 2020.2003.2012.988865, doi:10.1101/2020.03.12.988865 (2020). https://www.biorxiv.org/content/10.1101/2020.03.12.988865v2

9. Taiaroa, G. et al. Direct RNA sequencing and early evolution of SARS-CoV-2. bioRxiv, 2020.2003.2005.976167, doi:10.1101/2020.03.05.976167 (2020). https://www.biorxiv.org/content/10.1101/2020.03.05.976167v1

10. Denisa Bojkova, K. K., Benjamin Koch, Marek Widera, David Krause, Sandra Ciesek, Jindrich Cinatl, Christian Münch. SARS-CoV-2 infected host cell proteomics reveal potential therapy targets. Nature, doi:10.21203/rs.3.rs-17218/v1 (2020). https://www.researchsquare.com/article/rs-17218/v1 （我们 3 月 25 日简报有编译）

33. Bosch, B. J., Bartelink, W. & Rottier, P. J. M. Cathepsin L Functionally Cleaves the Severe Acute Respiratory Syndrome Coronavirus Class I Fusion Protein Upstream of Rather than Adjacent to the Fusion Peptide. Journal of Virology 82, 8887-8890, doi:10.1128/jvi.00415-08 (2008). https://jvi.asm.org/content/82/17/8887

39. Su, Y. C. et al. Discovery of a 382-nt deletion during the early evolution of SARS-CoV-2 bioRxiv, 2020.2003.2011.987222, doi:10.1101/2020.03.11.987222 (2020). https://www.biorxiv.org/content/10.1101/2020.03.11.987222v1

Abstract

Direct RNA sequencing using an Oxford Nanopore MinION characterised the transcriptome of SARS-CoV-2 grown in Vero E6 cells. This cell line is being widely used to propagate the novel coronavirus. The viral transcriptome was analysed using a recently developed ORF-centric pipeline. This revealed the pattern of viral transcripts, (i.e. subgenomic mRNAs), generally fitted the predicted replication and transcription model for coronaviruses. A 24 nt in-frame deletion was detected in subgenomic mRNAs encoding the spike (S) glycoprotein. This feature was identified in over half of the mapped transcripts and was predicted to remove a proposed furin cleavage site from the

S glycoprotein. This motif directs cleavage of the S glycoprotein into functional subunits during virus entry or exit. Cleavage of the S glycoprotein can be a barrier to zoonotic coronavirus transmission and affect viral pathogenicity. Allied to this transcriptome analysis, tandem mass spectrometry was used to identify over 500 viral peptides and 44 phosphopeptides, covering almost all of the proteins predicted to be encoded by the SARS-CoV-2 genome, including peptides unique to the deleted variant of the S glycoprotein. Detection of an apparently viable deletion in the furin cleavage site of the S glycoprotein reinforces the point that this and other regions of SARS-CoV-2 proteins may readily mutate. This is of clear significance given the interest in the S glycoprotein as a potential vaccine target and the observation that the furin cleavage site likely contributes strongly to the pathogenesis and zoonosis of this virus. The viral genome sequence should be carefully monitored during the growth of viral stocks for research, animal challenge models and, potentially, in clinical samples. Such variations may result in different levels of virulence and morbidity and mortality.

6. 冠状病毒的核糖核酸通过切割病毒的 polyU 序列进而帮助病毒逃避宿主的免疫监测

Coronavirus endoribonuclease targets viral polyuridine sequences to evade activating host sensors

来源：PNAS

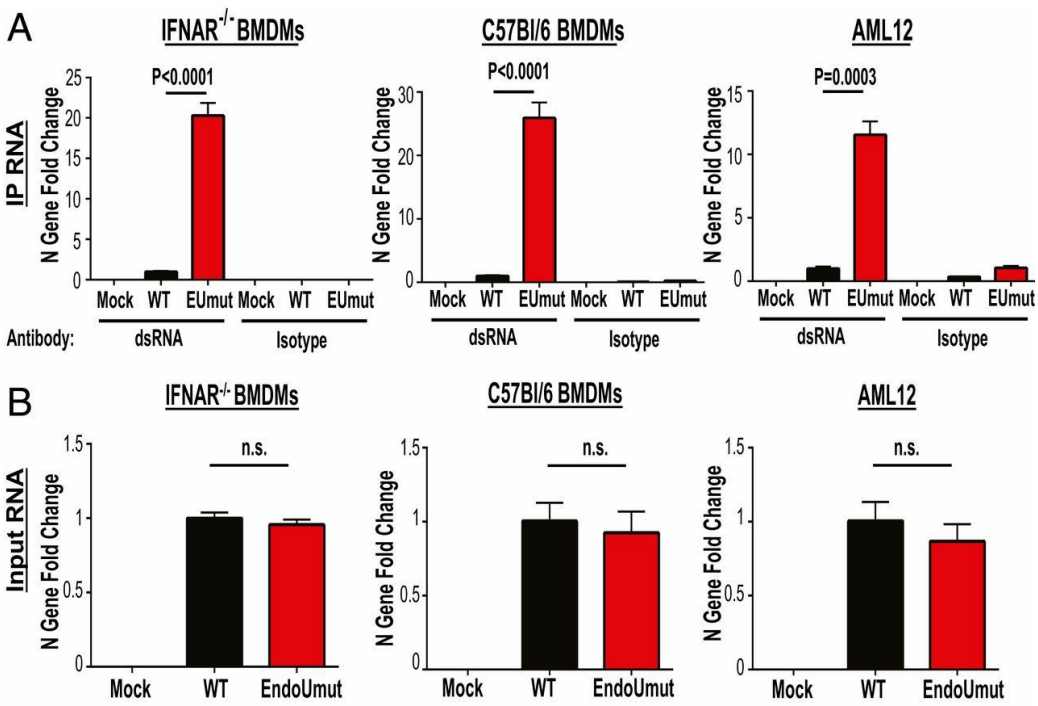
发布时间：2020-03-20

链接：https://www.pnas.org/content/early/2020/03/20/1921485117

细胞上带有对入侵病毒的监测器。为了避免被识别出来，冠状病毒会表达一些可以干扰宿主进行免疫识别的通路。以前的研究表明冠状病毒表达的核糖核酸内切酶 EndoU（在 SARS-CoV-2 中是 nsp15 基因）可以延缓宿主监测系统的激活。但是它的具体机制未知。这篇研究报道了 EndoU 通过切割病毒的 polyU 序列从而逃避宿主免疫监控。这个信息提示人们可以开发正对 EndoU 的抑制剂从而对抗冠状病毒。

这项研究揭示 EndoU 可以切割 polyA 病毒 RNA 为模板合成的病毒反义链 RNA 的 5‘端 polyU。相比野生型病毒感染的细胞，病毒反义链 RNA 的 polyU 浓度比在 EndoU 催化活性位点突变的病毒感染的细胞胞质中更高。用带 polyU 的病毒反义链 RNA 的转染细胞可以稳定激活 MDA5 依赖的干扰素反应。反之去掉 polyU 的病毒反义链 RNA 就不会激活宿主的 MDA5 依赖的干扰素反应。

总结来讲，这项研究表明带 polyU 的病毒反义链 RNA 是一个依赖于 MDA5 的冠状病毒病原相关分子模式（PAMP）。该研究揭示了冠状病毒 EndoU 通过切割破坏这个病原相关分子模式进而限制其累积。EndoU 的功能在冠状病毒里是高度保守，抑制它的活性有潜力成为干预已有以及新发冠状病毒的方法。



被识别双链 RNA 的抗体 K1 免疫沉淀下来的病毒 RNA 的定量分析

编者注：
病原相关分子模式（pathogen-associated molecular patterns PAMP）是模式识别受体（PRR）识别结合的配体受体，主要是指病原微生物表面某些共有的高度保守的分子结构。通常为病原微生物所特有，而宿主细胞不产生；为微生物的生存或致病性所必需；为宿主天然免疫细胞泛特异性识别的分子基础。
模式识别受体是一类主要表达于天然免疫细胞表面非克隆性分布、可识别一种或多种 PAMP 的识别分子（来源：百度百科）。

Cells carry sensors that are primed to detect invading viruses. To avoid being recognized, coronaviruses express factors that interfere with host immune sensing pathways. Previous studies revealed that a coronavirus endoribonuclease (EndoU) delays activation of the host sensor system, but the mechanism was not known. Here, we report that EndoU cleaves a viral polyuridine sequence that would otherwise activate host immune sensors. This information may be used in developing inhibitors that target EndoU activity and prevent diseases caused by coronaviruses.

Coronaviruses (CoVs) are positive-sense RNA viruses that can emerge from endemic reservoirs and infect zoonotically, causing significant morbidity and mortality. CoVs encode an endoribonuclease designated EndoU that facilitates evasion of host pattern recognition receptor MDA5, but the target of EndoU activity was not known. Here, we report that EndoU cleaves the 5′ -polyuridines from negative-sense viral RNA, termed PUN RNA, which is the product of polyA-templated RNA synthesis. Using a virus containing an EndoU catalytic-inactive mutation, we

detected a higher abundance of PUN RNA in the cytoplasm compared to wild-type-infected cells. Furthermore, we found that transfecting PUN RNA into cells stimulates a robust, MDA5-dependent interferon response, and that removal of the polyuridine extension on the RNA dampens the response. Overall, the results of this study reveal the PUN RNA to be a CoV MDA5-dependent pathogen-associated molecular pattern (PAMP). We also establish a mechanism for EndoU activity to cleave and limit the accumulation of this PAMP. Since EndoU activity is highly conserved in all CoVs, inhibiting this activity may serve as an approach for therapeutic interventions against existing and emerging CoV infections.
Overall, the results of this study reveal the PUN RNA to be a CoV MDA5-dependent pathogen-associated molecular pattern (PAMP). We also establish a mechanism for EndoU activity to cleave and limit the accumulation of this PAMP. Since EndoU activity is highly conserved in all CoVs, inhibiting this activity may serve as an approach for therapeutic interventions against existing and emerging CoV

7. 通过示踪 RNA 的合成揭示关于冠状病毒复制的统一结构和功能模型

A unifying structural and functional model of the coronavirus replication organelle: tracking down RNA synthesis
发布时间：2020-03-24
链接：https://www.biorxiv.org/content/10.1101/2020.03.24.005298v1
来自荷兰莱顿大学等团队最新的研究揭示了冠状病毒复制的统一结构和功能模型。
在被感染的细胞里，冠状病毒的 RNA 合成机器和被改变而转换为病毒复制细胞器的宿主细胞内质网质膜相关。虽然双层膜囊泡可能是冠状病毒通用的病毒复制单元，目前尚没有对其他类型由冠状病毒诱导的膜结构进行研究。尽管有一些猜测，研究者们仍然不了解病毒的 RNA 合成到底是在哪一类病毒复制单元中发生。
该研究通过对冠状病毒复制器进行 2 维和 3 维的分析，发现不同的冠状病毒基本上诱发同样的细胞膜结构修饰，包括之前前被认为只和 gamma 和 delta 冠状病毒感染相关、作为是 gamma 和 delta 冠状病毒的复制场所的小开口双层膜球。研究者们对新合成的病毒 RNA 进行了代谢标记，随后进行电子显微放射性定量分析，发现大量的病毒 RNA 合成和双层膜囊泡结构相关，在 beta 冠状病毒 MERS-CoV，SARS-CoV，以及 gamma 冠状病毒属的支气管病毒都是如此。RNA 合成和双层膜球以及其他细胞或者病毒诱导的结构都没有关系。这个研究揭示了一个通用的冠状病毒的复制器的模型，非常清楚地表明双层膜囊泡是病毒 RNA 合成的中心节点。这类双层囊泡可能是对抗冠状病毒的一个潜在药物靶点。

Zoonotic coronavirus (CoV) infections, like those responsible for the current SARS-CoV-2 epidemic, cause grave international public health concern. In infected cells, the CoV RNA-synthesizing machinery associates with modified endoplasmic reticulum membranes that are transformed into the viral replication organelle (RO). While double-membrane vesicles (DMVs) appear to be a pan-coronavirus RO element, studies to date describe an assortment of additional coronavirus-induced membrane structures. Despite much speculation, it remains unclear which RO element(s) accommodate viral RNA synthesis.
Here we provide detailed 2D and 3D analyses of CoV ROs and show that diverse

CoVs essentially induce the same membrane modifications, including the small open double-membrane spherules (DMSs) previously thought to be restricted to gamma- and delta-CoV infections and proposed as sites of replication. Metabolic labelling of newly-synthesized viral RNA followed by quantitative EM autoradiography revealed abundant viral RNA synthesis associated with DMVs in cells infected with the beta-CoVs MERS-CoV and SARS-CoV, and the gamma-CoV infectious bronchitis virus. RNA synthesis could not be linked to DMSs or any other cellular or virus-induced structure. Our results provide a unifying model of the CoV RO and clearly establish DMVs as the central hub for viral RNA synthesis and a potential drug target in coronavirus infection.