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Virus hunters: Discovering the evolutionary origins of SARS-CoV-2

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The likely animal source of SARS-CoV-2 remains speculative. A recent study published in Cell by Zhou et al. reported the detection of novel alpha- and betacoronaviruses, including SARS-CoV-2-related viruses in bats.

A new coronavirus (CoV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in 2019 to cause an unprecedented pandemic. While governments and communities are striving to control the ongoing coronavirus disease 2019 (COVID-19) pandemic, a question remains—where did SARS-CoV-2 come from? The clues perhaps lie in the documented evolutionary history of highly pathogenic betacoronaviruses (β-CoVs). In 2003, an outbreak of pneumonia was traced back to a new CoV, SARS-CoV (Drosten et al., 2003). Surveillance studies later identified a plethora of SARS-CoV-related coronaviruses (SARSr-CoVs) in Rhinolophus bats, suggesting that bats were the likely source of SARS-CoV (Li et al., 2005). Finally, the discovery of a SARSr-CoV in Himalayan palm civets that was 99.8% identical to SARS-CoV established the likely transmission route of SARS-CoV from palm civets to humans (Guan et al., 2003). Similarly, the discovery of Middle East respiratory syndrome coronavirus (MERS-CoV) in dromedary camels, along with detection of MERS-CoV-like viruses in bats established the likely evolutionary trajectory of MERS-CoV from bats to camels to humans. It is paramount that we confidently identify the source of SARS-CoV-2 to prevent a similar outbreak in the future.

Identifying the wildlife reservoir host of a zoonotic virus is a Herculean task. Zoonotic transmission events are opportunistic and transient (Plowright et al., 2017), making them difficult to identify or trace. The first step to identify a wildlife reservoir of a pathogen is to detect or, preferably, isolate the pathogen from that animal. In a recent study published in Cell, Zhou et al. (2021) collected 411 bat samples from an ecologically rich area in Yunnan province, China between May 2019 and November 2020 to investigate the natural existence of SARS-CoV-2-related viruses (Zhou et al., 2021).

Zhou and colleagues used meta-transcriptomics to sequence total RNA extracted from the bat samples (Figure 1). This process led to the discovery of 9 sarbecoviruses (subgenera or lineage containing SARSr-CoV) and 17 alphacoronaviruses (α-CoVs). Of note, 4 out of 7 novel sarbecoviruses (RpYN06, RsYN04, RmYN05, and RmYN08) were closely related to SARS-CoV-2, while the remaining 3 (RsYN03, RmYN07, and RsYN09) were more closely related to SARS-CoV (Figure 1A). Interestingly, all novel sarbecoviruses were detected in bats from the genus Rhinolophus. At the whole genome level, one of the novel sarbecoviruses, RpYN06, was 94.48% identical to SARS-CoV-2 (Zhou et al., 2021), making it the second closest known ancestor of SARS-CoV-2 after RaTG13 (96.1%) (Zhou et al., 2020) (Figure 1B). RpYN06 exhibited only 76.33% nucleotide identity to SARS-CoV-2 within the spike gene and only 60.91% in the receptor-binding domain (RBD); however, for some individual genes, such as ORF1ab, ORF7a, ORF8, N, and ORF10, RpYN06 exhibited the highest known sequence identity to SARS-CoV-2 to date (Figure 1B). RsYN04, RmYN05, and RmYN08 exhibited over 99.96% nucleotide identity to each other, highlighting the close interspecies interaction and virus transmission between Rhinolophus stheno and Rhinolophus malayanus. In phylogenetic analysis, RsYN04, RmYN05, and RmYN08 clustered with pangolin-derived CoVs from Guangxi, albeit they were evolutionarily distantly related. Perhaps there is still a missing link between bat-borne and pangolin-borne CoVs.

In addition to β-CoVs, Zhou and colleagues identified 17 novel α-CoVs. Of these, MIYN15 and RsYN25 detected in Myotis laniger and R. stheno were closely related to swine acute diarrhea syndrome CoV (SADS-CoV), a recently discovered CoV that causes acute diarrhea in young pigs (Zhou et al., 2018). HtYN18, detected in a Hipposideros larvatus, was closely related to the porcine epidemic diarrhea virus (PEDV). Furthermore, the study also identified novel, unclassified CoVs in bat specimens, including a possible novel species of subgenus Decacovirus. The study by Zhou et al. (2021) clearly demonstrates that in spite of almost two decades of research on bat-borne CoVs, we have only scratched the surface of the true diversity of CoVs in bats. The order Chiroptera is made up of over 1,400 different species, and the vast majority of them have not been studied.

To facilitate cross-species transmission into humans, zoonotic CoVs need to interact with human cell surface receptors to facilitate cellular entry and virus replication. The RBD within the spike protein makes critical contacts with the human cellular receptor, angiotensin-converting enzyme 2 (ACE2), to facilitate virus entry. At the six spike amino acid residues that have been deemed critical for binding to ACE2, SARS-CoV-2 and the three bat-borne viruses (RsYN04, RmYN05, and RmYN08) only shared two amino acids—L455 and Y505 (Zhou et al., 2021). RpYN06 only contained one
identical amino acid with SARS-CoV-2 in the RBD—Y505. Zhou et al. (2021) utilized binding assays to demonstrate that RsYN04 RBD could weakly interact with human ACE2, whereas RpYN06 RBD did not, suggesting that RsYN04 could potentially infect human cells (Figure 1A). The role of L455 and Y505 in facilitating virus entry and replication in bat and human cells remains unidentified.

A separate study investigating ACE2 orthologs from 46 bat species demonstrated that wild-type SARS-CoV-2 is able to selectively utilize 25 of 46 bat ACE2 orthologs for cellular entry, while 21 of 46 bat ACE2 orthologs did not support virus entry (Yan et al., 2021). In a complementary study with another bat-borne CoV, RaTG13, Liu et al. (2021) demonstrated that the RBD of RaTG13 could weakly interact with ACE2 orthologs from multiple mammalian species, suggesting that this virus is likely capable of entering cells from these mammals. Liu et al. (2021) identified residue D501 in RaTG13 RBD as a key amino acid that may facilitate bat-intermediate-host adaptation; however, the amino acid residue at this position in RpN06, RsYN04, RmYN05, and RmYN08 RBD is valine, which raises intriguing questions regarding the zoonotic potential of these newly identified viruses (Zhou et al., 2021). The furin cleavage site in SARS-CoV-2 spike protein is cleaved by cellular enzymes to facilitate infection. Of note, none of the four SARS-CoV-2-related viruses discovered in the study by Zhou et al. (2021) contained the furin cleavage site (PRRAR) or the proposed

Figure 1. Key findings from Zhou and colleagues’ article
(A) Schematic representation of the study design and key observations. Zhou et al. (2021) identified 17 alphacoronaviruses and 7 novel betacoronaviruses in 411 bat samples collected from Yunnan province, China. Four of seven detected betacoronaviruses were closely related to SARS-CoV-2, while the remaining three were related to SARS-CoV. RsYN04 receptor-binding domain (RBD) displayed weak binding affinity to human cellular receptor, angiotensin-converting enzyme 2 (ACE2). The RBD from betacoronavirus RpYN06 did not bind to ACE2. The newly discovered alphacoronavirus sequences included swine acute diarrhea syndrome CoV (SADS-CoV)-like and porcine epidemic diarrhea virus (PEDV)-like viruses.

(B) Nucleotide identity of two bat-borne betacoronaviruses, RaTG13 and RpYN06, compared to SARS-CoV-2 (reference sequence: NC_045512). Whole-genome and gene-level percentage nucleotide identity reported by Zhou et al. (2021) are shown here. Figure was made using BioRender.com and Adobe Illustrator (v25.3).
furin landing site (QQTQNS). Further studies are required to characterize the interaction of bona fide bat-borne CoVs with cells derived from their specific bat hosts.

Multiple bat species can roost together, facilitating the transmission and recombination of bat-borne CoVs. Previous studies have demonstrated that nutritional and reproductive stress can lead to increased Hendra virus replication in Pteropus scapulatus (Plowright et al., 2008). Using ecological measurements, Zhou et al. (2021) speculate that temperature seasonality, evapotranspiration, and continentality could affect the distribution of bat species. Most importantly, the authors identified a high richness of rhinolophid bats across much of Southeast Asia and southern China. This high density of Rhinolophus bats could potentially facilitate intra- and interspecies transmission and recombination of bat-borne sarbecoviruses. As sampling of bat populations and additional wildlife species intensifies, research will highlight the true diversity of CoVs that exist in our wildlife population.

While debate on the origin of SARS-CoV-2 continues, this recent study by Zhou et al. (2021) further bolsters the natural existence of SARS-CoV-2-related viruses in Rhinolophus bats.

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Beyond neutralization for BNT162b2 mRNA vaccination

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Mounting a robust immune response against SARS-CoV-2 requires neutralization as well as effector T cell functions. In this issue of Cell Host Microbe, Tauzin et al. characterize the humoral and T cell responses after a single dose of BNT162b2 mRNA vaccine in individuals with or without previous exposure to SARS-CoV-2.

We are entering into the 18th month of the ongoing global severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic that has claimed over 3.8 million reported deaths. As of May 2021, over 600 million people have been at least partially vaccinated against coronavirus disease 2019 (COVID-19), yet billions are still left vulnerable without access to an approved vaccine worldwide. We are facing unprecedented times where more than 6 billion people will require immunization against this virus. If two or multiple booster doses (against potential new variants) are required, this