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Global Pandemic Preparedness: Why the Origin of SARS CoV-2 is Important

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Abstract: Preparing for the next global pandemic has become much more challenging given the probability that SARS CoV-2, the virus that causes COVID-19, was engineered. Recent investigations on the origin of the virus have focused on human intelligence findings, but human intelligence is notoriously fickle and easy for a skilled actor to manipulate. If the chain of evidence that would show that human engineering was responsible for the origin of SARS CoV-2 has been destroyed, the current investigations may hit a dead end. If that is the case, then understanding the evidence presented by the virus itself is critical to show the strong possibility that human agency was responsible for the COVID-19 pandemic. If humans are responsible for the COVID-19 pandemic, then how must we all prepare for the next pandemic?

Introduction: A former US Senator, George Allen, observed that understanding the origin of SARS CoV-2 is important for several reasons[1]. Perhaps the two most important reasons are:

1. we must prepare for the next global pandemic and
2. understanding the nature of threat is vital to our preparedness.

There are three possibilities here.
First, the pandemic had a natural origin, similar to all previous pandemics.
A second possibility is that the virus had a natural origin but escaped from a laboratory. The third alternative is that SARS CoV-2, the virus that causes COVID-19, was engineered.
How the US and the rest of the world prepares for the next pandemic hinges upon this question.

- If the virus has a natural origin, then stepping up surveillance of potentially zoonotic viruses is called for.
- If the virus was engineered, then much more careful monitoring of labs working with viruses is called for. Furthermore, novel mechanisms of action of these viruses may pose additional challenges.

Recent publicly available intelligence community assessments of the origin of SARS CoV-2 have concluded that the virus probably does not have a man made origin. While two agencies disagreed and separately concluded that the virus did have human input in its development, the agencies had low confidence in this conclusion[2].

These intelligence community estimates are deeply flawed. The likely reason is that many publications in the scientific literature around the origin of SARS CoV-2 have contorted their conclusions to support the idea of natural origin. Often this conclusion is at odds from the data

[1] Former Senator Allen made these comments during a Nanobusiness Commercialization Association Zoom call in March, 2023.
[2] https://www.dni.gov/files/ODNI/documents/assessments/Unclassified-Summary-of-Assessment-on-COVID-19-Origins.pdf

presented in the paper, such as a novel mechanism of replication which leads to the reasonable question: could the virus have been engineered? By not only failing to address the possibility that the virus was engineered, but often even denying it, the reader is left befuddled, and may incorrectly assume that they have not interpreted the findings in the paper correctly. Members of the intelligence community rely on the accuracy of the peer reviewed literature as part of the information gathering process. Whether the intelligence community has sufficient scientific depth to understand the nature of systematic flaws in the peer reviewed literature is an open question. Based on what's happened, if the science community makes a mistake, then the intelligence community is unlikely to detect error. This is a bold claim, but unfortunately, the intelligence communities largely tacit acceptance of the natural origin of SARS CoV-2 suggests that this is likely the case.

The global pandemic of COVID-19 is an "emperor has no clothes" story. Respected members of the scientific community continue to advance a theory which is becoming increasingly far-fetched, i.e., that SARS CoV-2 has a zoonotic (natural) origin, rather than having engineered components[3]. Publications ignore key pieces of data to continue to promulgate the hypothesis of a natural origin of SARS CoV-2[4]. It's become a near-religious dogma and opposing papers are discouraged[5]. At a minimum, given the unusual findings surrounding SARS CoV-2, both a natural origin and the possibility of a lab leak should be considered as viable explanations for how this virus came to be, and its impact on the globe. More likely, SARS CoV-2 was engineered in a laboratory.

The Origin Issue:

There are four questions that need to be considered here:

1) SARS CoV-1 originated in bats and then spread to humans. SARS CoV-2 also originated in bats and then spread to humans. Why do the two viruses have such different mechanisms of replication?
2) SARS CoV-2 has a complex mechanism of replication that has not been seen in other viruses to date. How did this mechanism arise?
3) SARS CoV-2 has some unusual characteristics that suggest a different evolutionary pathway than SARS CoV-1. These characteristics of SARS CoV-2 include a ready and rapid adaptation to humans and an inability to reproduce in the original species, i.e., bats. Why?
4) The genetic codons that are the "blueprint" for the replication of the SARS CoV-2 contain both human and bat codons, but only in a novel region of the virus, i.e., the furin cleavage site. SARS CoV-1 only has bat codons. Why?

Based on these questions, there is no sound reason to exclude the possibility that SARS CoV-2 was genetically engineered, yet most scientific publications on SARS CoV-2 hew to the dogma

[3] https://www.science.org/doi/10.1126/science.abp8715
[4] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8448488/
[5] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8316641/

that the virus has a purely natural origin and dismiss out of hand the possibility that the virus was engineered. Most scientists do accept that bioengineering is now capable of making the needed changes in a bat coronavirus to turn it into SARS CoV-2 without leaving tell tale traces- they're just saying it didn't happen.

The story of human activities around SARS CoV-2 can be compelling[6], but so is the evidence from the virus itself. The advantage of disregarding the chain of coincidences involving humans such as a laboratory studying bat coronaviruses located in the city where SARS CoV-2 sprang from and instead focusing solely on what is known from the virus itself is objectivity. Humans can lie- the virus cannot. While there's a lot of smoke and mirrors here, the fundamental issue isn't hard to understand: it's timing. All of the steps that are needed to produce SARS CoV-2 from a bat coronavirus can happen in nature and there have been plenty of examples that have been trotted out. The key question though is, could all of those steps happen so quickly? The fundamental difference between a natural origin of SARS CoV-2 and an engineered version is timing.

In nature, viruses have two ways that they can change over time. RNA viruses, like SARS CoV-2 or influenza, have an appreciable mutation rate which is a function of how the virus replicates. This mutation rate improves the fitness of the virus relatively slowly, since it works by random selection, i.e. there's a change in the genetic code of the virus which can accomplish one of three things:

A) Improve the replication of the virus.
B) Do nothing.
C) Decrease the replication of the virus.

Natural selection eliminates option C, so when a mutation happens, after a period of time, it either improves the fitness of the virus or does nothing. Most of the time, nothing significant happens.

There's a second way that viruses can change though. Rather than change one letter of the genetic code at a time, the virus can swap bigger parts of the genome with another virus[7] which can happen when two viruses invade the same cell. Luckily, this simultaneous infection happens infrequently because when it does happen, the virus can gain functionality quickly- like being able to jump into another species. There's an important limitation here: the virus can only swap pieces of genetic code that already exist- the virus can't come up with new code, it can only rearrange what its starting with.

When scientists want to alter the function of a virus, they don't have to wait for random mutations to occur, they can "borrow" pieces of genetic code from other viruses or they can come up with their own. Thus, human modification of viruses is a lot faster than what nature can do. Humans can do "intelligent design", nature can't.

[6] Here are two: www.pnas.org/doi/abs/10.1073/pnas.2202769119, https://thebulletin.org/2021/05/the-origin-of-covid-did-people-or-nature-open-pandoras-box-at-wuhan
[7] https://pubmed.ncbi.nlm.nih.gov/11732610/

The Evidence For a Zoonotic Origin of SARS CoV-2

All pandemic viruses prior to SARS CoV-2 arose zoonotically. In other words, the virus evolved in a host animal species and then managed to jump into humans. The jump into humans can happen directly from the original species harboring the virus (mosquitoes and ticks are really good at transmitting viruses or other infectious species to humans directly) or it can involve an intermediate species (e.g, camels, as seen in MERS). This long history of zoonoses does provide a powerful argument for a natural origin of SARS CoV-2, but it is not sufficient. Nor should this argument blind us from objectively considering the possibility that SARS CoV-2 was an engineered virus.

There are three requirements a virus has to fulfill to cause a pandemic.

1) It has to be able to jump into humans efficiently. If the virus can't do this, it can't infect many humans.
2) The virus has to have efficient human to human transmission or a ubiquitous intermediate such as a mosquito.
3) Humans can't have pre-existing immunity to the virus.

Looking at other recent (last two decades) lethal coronaviruses, SARS CoV-1 and MERS, there are clear differences between the two. Let's look at MERS first because it's a bit simpler. Both coronaviruses likely originated in bats, but for MERS, the virus evolved to jump into camels prior to making the jump to humans. Once it made the jump to humans, it still couldn't transmit from human to human. The virus is so highly pathogenic (~1/3rd mortality rates), it is unlikely to progress further in the human host because it needs a living host to be able to evolve. When the host dies, that kills the virus too, so from the perspective of the virus, MERS is probably a dead end. Since camels aren't that widely geographically distributed, most of the planet doesn't have to worry about the virus, unless of course it mutates in camels to allow human to human transmission. So far that hasn't happened.

SARS CoV-1 originated from what is still an unknown bat species[8]. It turns out that the location where the virus evolved may be more important than in which species, (see below) but there is still a critical gap. There are three key pieces of information needed to understand SARS CoV-1: the bat species, the original virus, and where it happened. There's been a lot of debate as to how SARS CoV-1 evolved and it's very relevant to understanding the development of SARS CoV-2 as there are important similarities and differences between the two viruses. A key aspect to the discussion is how viruses enter cells.

For a virus to get into a host cell- it has to be able to bind to that cell, otherwise, it'll just get swept away. Viruses have no way to move on their own, so where the virus binds has got to be pretty sticky. The site on the cell membrane that several coronaviruses (SARS CoV-1, SARS CoV-2 and NL63- a cold virus) bind to is called ACE2 (angiotensin converting enzyme 2). In a

[8] www.ncbi.nlm.nih.gov/pmc/articles/PMC7323513/

multicellular organism, cells have to be able to communicate with each other and the ACE2 site has a number of important functions, but admitting viruses isn't one of them. Essentially, viruses hijack the cellular communication network in an organism for their own nefarious purposes.

For a coronavirus to bind to an ACE2 site, the virus has to have a piece of "hook" material the correct shape to match the "loop" material on the host site- it has to be sticky like Velcro. This location where the virus binds (i.e., analogous to the hook material) is called the receptor binding domain of the virus. If the virus's receptor binding domain has the wrong shape (amino acids that don't match what's needed for the binding site[9]) the virus just doesn't stick well and never enters the cell. When a virus jumps to a new species- this step is critical. If the binding site of the host animal that the virus is in originally isn't pretty similar to the binding site in the new host species– the virus can't make the jump.

There's a lot of variability in bat ACE2 sites. This wasn't well known when SARS CoV-1 jumped into humans 20 years ago. The first bat ACE2 sites scientists found looked pretty different from human ACE2 sites, they shared only 1 out of the 5 or 6 of the amino acids in the receptor binding site. That would mean that the bat viruses could never jump into humans because the ACE2 sites between the species are so different. Scientists proposed that SARS CoV-1 had to go through a process like MERS did to get into humans, i.e., an intermediate species. There are numerous papers in the literature that discuss which potential species were involved in the original bat coronavirus making the jump into humans, but the exact pathway and the original bat coronavirus species have never been identified. Nor has the original bat coronavirus been identified for MERS. Identifying these original bat coronaviruses is not so easy to do.

In 2017, a group that had been studying bat coronaviruses published some interesting discoveries[10]. What they found in a single cave in Yunnan, China was that bat coronaviruses were really pretty promiscuous- various receptor binding domains were shared between various viruses. There were several species of bats present, the coronaviruses had evolved to infect them all. What this shows is that bat coronaviruses can swap key pieces of genetic material when in close geographic proximity. While these types of genetic swaps are rare events, since there are so many bats living so close together, the odds of these swaps happening increased.

Some of the bat coronaviruses in this cave in Yunnan had receptor binding domains that worked well in humans. The scientists were unable to determine which coronavirus had evolved to SARS CoV-1, but all the critical pieces were there. Hence, no need for an intermediate species- the likely path of transmission of SARS CoV-1 went directly from bat to human. Since the original viral progenitor is still unknown, yes, there still could have been an

[9] Amino acids are the building blocks of proteins, and these components, the receptor binding site and the receptor binding domain on the virus are both proteins.
[10] https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1006698

intermediate species similar to what has been seen in MERS, but it's not a requirement and it has never been found.

That SARS CoV-1 evolved to jump from bats to humans does provide some support for a natural origin of SARS CoV-2 as there's no need to invoke either another species or human engineering. However, the binding receptor site wasn't the only region of SARS CoV-2 that needed to change to infect humans so quickly, there's a furin cleavage site as well. (See below.)

As an aside- the oft floated idea that SARS CoV-1 jumped from carnivores such as pangolins or racoon dogs into humans is on some shaky ground. Some evolutionary virologists have concluded that the transmission went the other way around[11]. In other words, similar to what has been seen with SARS CoV-2 where humans have transmitted the virus to other species[12], SARS CoV-1 also jumped from humans into the carnivores. What is clear though, is that a bat coronavirus somehow made the jump into humans some 20 years ago which led to SARS CoV-1. Luckily the virus wasn't that great at human-to-human transmission and the epidemic burned out relatively quickly.

The Evidence Against the Zoonotic Origin of SARS CoV-2

All organisms have an evolutionary "clock". If you can identify the progenitor species of a plant, an animal or a virus and then determine the percent of homology- that's the number of similar letters in the genetic code between the two species, you have a rough idea of how much time has passed from an evolutionary standpoint. Genetic sequences that have a high degree of homology haven't had much time to drift apart, while sequences that have less homology have diverged over many generations. The degree of homology doesn't necessarily indicate a change in structure though. Lots of changes in the genetic code have no real effect, the overall shape of the protein coded by the piece of DNA or RNA that's altered doesn't change. This is why biologists can identify species over time even if their genetic code has changed dramatically, i.e., less than 20% homology. If the shapes of the proteins specified by the genetic code haven't changed and neither has their function, well, that's the same species. SARS CoV-1 shares about 92% of its genetic identity with other bat coronaviruses[13], while SARS CoV-2 shares over 96% with the bat coronavirus RaTG13.

In contrast to SARS CoV-1 where there is no certain identity of the originating bat coronavirus, the bat coronavirus which led to SARS CoV-2 was RaTG13- a horseshoe bat coronavirus. This is based on the homology that SARS CoV-2 has with RaTG13.

Understanding the import of this amount of homology is critical to understanding what has taken place. The fact that there's a several percent difference means that there has been some

[11] https://onlinelibrary.wiley.com/doi/full/10.1111/cla.12425
[12] The use of the term species can be confusing. The family of bats contains numerous bat species, but humans are only a single species.
[13] www.annualreviews.org/doi/10.1146/annurev-virology-100114-055029

evolutionary time that's passed. Bat coronaviruses have much higher percentages of homology with each other: around 99.8%. That's close- minimal adaptation time needed as the virus is jumping into very similar species. After a virus jumps into a different family though, it needs to adapt. As noted above, SARS CoV-1 has ~ a 92% homology with other bat coronaviruses. If RaTG13 is the progenitor of SARS CoV-2, then there hasn't been a lot of evolutionary time for SARS CoV-2 to develop on its own, it's way too close to its parent. SARS CoV-2 though, clearly didn't come straight from a bat, there's too much evolutionary time that's passed. As a reference point; in terms of the similarities between the coronaviruses, MERS shares about 50% of its genetic material with SARS CoV-2, while SARS CoV-1 shares about 80% of its genetic material with SARS CoV-2.

Summarizing all this data:

1) SARS CoV-1 went straight from a bat to a human and has remained close to its bat ancestor with ~92% similarity.
2) SARS CoV-2 did NOT go from a bat into a human- it needed an intermediate species, and yet has remained even closer to its bat ancestor than SARS CoV-1.
3) MERS also needed an intermediate species and evolved further (less similarity to its original bat ancestor virus) than either SARS CoV-1 or SARS CoV-2.

How did SARS CoV-2 get into humans so quickly?

Viruses have to adapt quickly to their new hosts. If a virus can't efficiently reproduce in the new host, it dies out, so when a virus is first introduced to a new host, there are a lot of changes that occur quickly, at least that is what has been seen in other viruses. SARS CoV-2 however, is an exception. The virus already seemed to be pretty well adapted to humans as the pace of change when it was first discovered has been quite leisurely[14], especially compared to SARS CoV-1[15]. With SARS CoV-1, there were up to 85 substitutions in the amino acids of the virus in the early to mid phase of the pandemic (first three months from January to March), while there were only only 15-25 substitutions in SARS CoV-2 during a similar time period. That slower pace of substitutions is a lot closer to the pace seen in SARS CoV-1 after it had been in humans for a few months. Why didn't SARS CoV-2 have to go through a similar rapid period of adaptation as SARS CoV-1?

There are two critical changes that the bat coronavirus, RaTG13, has to undergo to become SARS CoV-2. First, the receptor binding domain has to be dramatically altered. The receptor binding domains in RaTG13 and SARS CoV-2 aren't close- they only share one of the 6 needed amino acids. Based on the recent findings of bat coronaviruses from the cave in Yunnan, this step may not be so unlikely as other bat coronaviruses have receptor binding domains that are a better match with human ACE2. Given that bat coronaviruses do trade these receptor binding domains via genetic recombination, a natural origin for the receptor binding domain in SARS CoV-2 is perfectly reasonable.

[14] https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3001115
[15] www.biorxiv.org/content/10.1101/2020.05.01.073262v1.full.pdf

The second critical change that has to happen to RaTG13 is the addition of a furin cleavage site (FCS) in the spike protein. Furin cleavage sites signal a common enzyme, known as furin, to make a cut in the protein at or near this location. Since enzymes (or proteases) are powerful ways to manipulate proteins, mother nature has found a way to ensure that these enzymes are used only where needed. In the target protein, there's a string of amino acids that the protease recognizes known as a cleavage site. It's a very similar idea to the receptor binding domain. When the protease "sees" this cleavage site, the protease is activated and it cuts the protein. Furin cleavage sites are a feature that's not found in SARS CoV-1 nor other bat coronaviruses, but it is found in MERS and some other coronaviruses. It's really important to understand what that site is doing and how it got there because it's critical to understanding how SARS CoV-2 infects cells. While SARS CoV-1 has a similar mechanism of action to other coronaviruses, SARS CoV-2 has a different mechanism than any other coronavirus that's been found: SARS CoV-2 is unique- because of this furin cleavage site. The furin cleavage site[16] that's present in SARS CoV-2 dramatically changes the mechanism of how the virus replicates (see below). Doesn't that suggest that the virus should have to make lots of changes when it gets into humans? Yes, the changes could have taken place in the putative intermediate species, but the clock is ticking.

There's another issue here around adaptation of viruses. When one virus hops into another species, it can still infect its previous host species. That's because the virus still needs the host species to make lots of copies to spread to the new species and when the virus replicates in the new species, it can still infect the original host species. SARS CoV-1 can still infect bats. SARS CoV-2 does a really lousy job of that[17]. That's why SARS CoV-2 needs an intermediate species to get from bats to humans. Yes, if there's an intermediate species, that might explain all the changes seen in SARS CoV-2 from the original bat species. The problem here is again the ticking clock of homology- how does SARS CoV-2 not drift very far from the original bat species and yet be unable to infect bats well? The reason SARS CoV-2 doesn't infect bats appreciably is the furin cleavage site.

The presence of the furin cleavage site in the spike protein of SARS CoV-2 is really difficult to explain. It's clear that it's a recent addition (not seen in other bat coronaviruses) and adding the furin cleavage site makes it harder for the spike protein to do its job in bats. That's why the explanation is difficult. If the furin site were added in a bat, it would get "spit out" in short order. In other words, other versions of the virus that didn't have the furin cleavage site would outcompete the ones that did. So the furin cleavage site didn't get added to the virus when it was in a bat. There's been some really convoluted logic that claims that because the furin cleavage site gets spit out when the virus replicates in human vero E6 cells in the laboratory, that's evidence of a natural origin. This is baffling, because the furin cleavage site makes SARS CoV-2 more infectious in humans than SARS CoV-1- that's why the furin cleavage site doesn't get spit out when the virus infects humans. The furin cleavage site provides an important

[16] The furin cleavage site is ~ ½ dozen amino acids that signal to the furin cleaver enzyme to cut here. Furins are ubiquitous cleaver enzymes.
[17] www.ncbi.nlm.nih.gov/pmc/articles/PMC7706959/

evolutionary advantage to the virus, it helps the virus replicate more efficiently in humans- but not in bats.

The spike protein's job is to get the virus into the cell. See Figure 1: which shows the replication of SARS CoV-1 and SARS CoV-2. The spike protein has a complex, dynamic structure- it's far from static. There are a number of steps to get the virus into the cell, which of course has a number of defenses to prevent the virus doing what it's attempting, so the spike protein has to go through several different configurations. Spike proteins are highly adaptable- they change depending upon what cell they're trying to enter[18]. When SARS CoV-2 tries to replicate in bats- the furin cleavage site makes several needed configurational changes energetically unfavorable which is why SARS CoV-2 doesn't replicate well in bats. What this means is that SARS CoV-2 requires an additional species to get to humans which is in striking contrast to SARS CoV-1 which doesn't. Was that additional species a mouse with a humanized ACE2 site being raised in a lab or was it another species in the wild, as yet unknown? There's been an exhaustive search for this species in the wild, so far, it's turned up nothing[19].

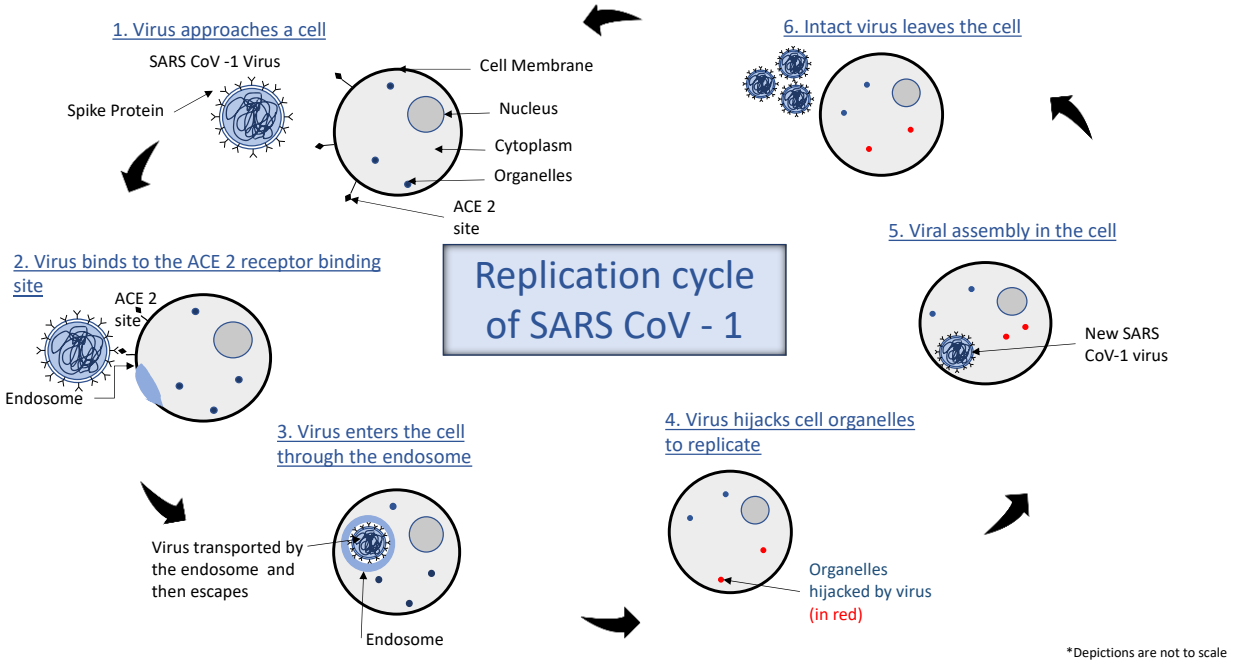
For some coronaviruses, the first step for the spike protein to enter the cell involves cutting a covalent bond (that's a strong chemical bond) to break the spike protein into two pieces, S1 and S2. This step doesn't always happen- for example, this cut may not occur in SARS CoV-1. The S2 piece stays attached to the rest of the virus with a covalent bond, but the S1 piece now stays attached with bunch of weaker hydrogen bonds. It helps make the active site accessible and to allow the spike protein to fold correctly. Obviously in bats, the furin cleavage site isn't needed for this step as the virus does fine without it. The virus also doesn't need this furin cleavage site to get into human cells either, as shown by SARS CoV-1. But what would happen if a furin cleavage site would be added to a spike protein is an interesting question for virologists and there's been a lot of speculation over the years[20]. Adding a furin cleavage site to spike proteins of coronaviruses was first successfully done in Jack Nunberg's lab in 2006[21] at what was thought to be the S1/S2 cleavage site. Other groups also added furin cleavage sites to the spike protein in SARS CoV-1[22].

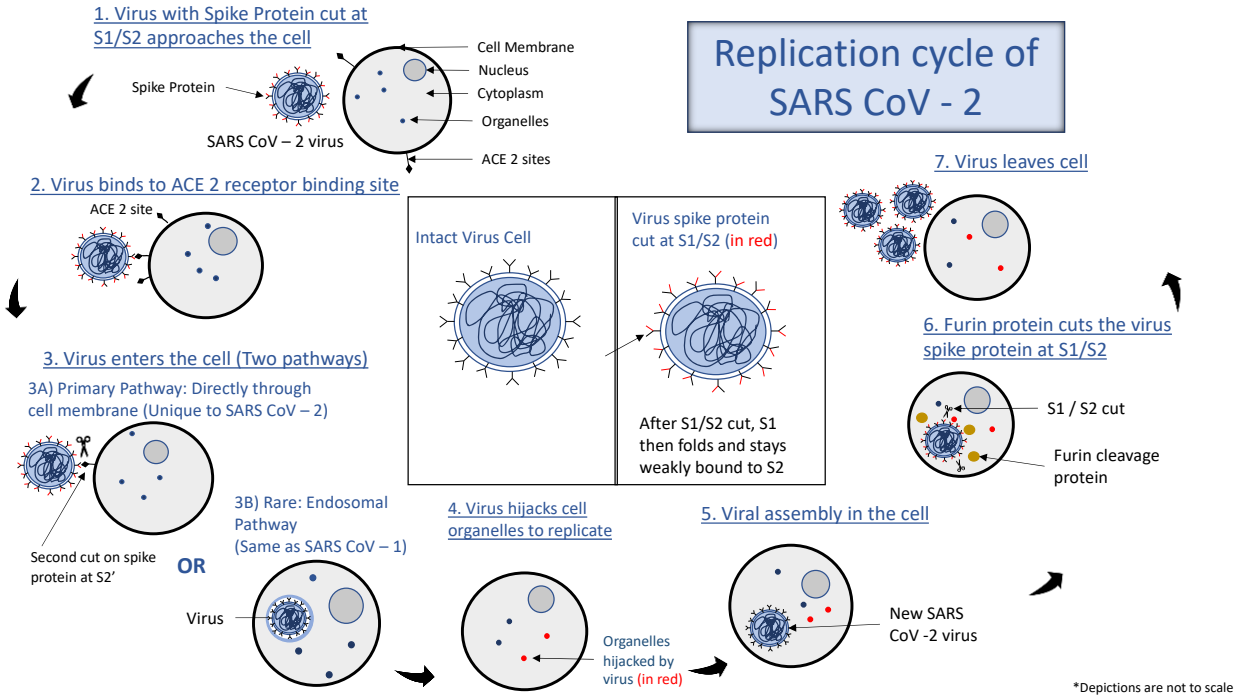
The addition of the furin cleavage site at the S1/S2 site in the spike protein of SARS CoV-2 radically changes the mechanism of how the virus replicates in human cells compared to SARS CoV-1- and all other coronaviruses[23].[24]. The furin cleavage site in SARS CoV-2 leads to the spike protein being cut at S1/S2 while the virus is still in the cell that produced it![25] This step normally happens when the virus approaches the cell it's trying to get into- not before it's released from the cell which made it. When SARS CoV-2 comes into contact with the host cell,

[18] www.sciencedirect.com/science/article/pii/S187962572100016X
[19] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8448488/#bib13
[20] https://academic.oup.com/mbe/article/39/1/msab327/6426085
[21] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8689951/#msab327-B13
[22] https://pubmed.ncbi.nlm.nih.gov/19321428/
[23] www.nature.com/articles/s41580-021-00418-x
[24] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8689951/
[25] https://onlinelibrary.wiley.com/doi/full/10.1111/1348-0421.12945

the first step is now a second cut at the S2 protein referred to as S2'. Once this second cut is made by the trans membrane serine proteases (aka TMPRSS2- a protease whose function is not well understood), then SARS CoV-2 can enter the cell in a very different manner than other coronaviruses. Let's be clear. Furin cleavage sites have been found in other coronaviruses spike proteins- that's not the issue. The problem with the furin cleavage site in SARS CoV-2 is *where* it is in the spike protein which hasn't been seen before- and the composition of the furin cleavage site. (See below.)

Figure 1: The Replications cycles of SARS CoV-1 and SARS CoV-2





Source: Nanobiz, LLC

All other coronaviruses enter the cell through an endosome- it's a compartment the cell has to transport what's outside the cell inside. You can think of it as dumbwaiter for the cell except it's got sentries to check what goes in and what comes out. Viruses have developed lots of tricks to escape from the endosome into the cytoplasm as the cytoplasm is where the virus coopts the cells organelles into making virus reproduction factories. SARS CoV-2 doesn't need to use these tricks to escape the endosome as once the S2' cut has been made, the virus can fuse directly with the cell membrane and get into the cytoplasm directly- without going through the endosome! If the cell blocks SARS CoV-2 from entering the cell through the membrane, well, SARS CoV-2 can still use the endosomal pathway like other coronaviruses. In short, SARS CoV-2 has a very different mechanism of replication compared to any other coronavirus we've seen. As an aside, this different mechanism of entry is why drugs like chloroquine/hydroxychloroquine failed- they were blocking a mechanism that the virus didn't use.

As noted above, there's another issue with the furin cleavage site in SARS CoV-2- the sequence of the furin cleavage site hasn't been seen before in other viruses. Furin cleavage sites have some variability- they have to signal the furin protease to make a cut, but there's a range of signaling options. The virus doesn't have to get a specific sequence exactly correct, close enough will do.. The amino acid sequence of the furin cleavage site in SARS CoV-2 is four amino acids: proline-arginine-arginine-alanine. (often written: P-R-R-A) The neighboring arginines are a tip off that something is awry- arginines are almost never found paired this way in nature. If SARS CoV-2 is going to rapidly acquire a furin cleavage site- where did it come from?

The genetic coding for the amino acids of the furin cleavage site in SARS CoV-2 doesn't match other coronaviruses[26]. A brief recap of how amino acids in proteins gets specified may help here. Nature uses groups of three bases (out of the 4 letters of the tRNA genetic code: A, G, U, and C) termed a codon to specify which of 20 amino acids is needed. There is redundancy in this system as there are 64 possible codons, but only 20 amino acids. Obviously, some amino acids can be specified by more than one codon, but typically, an organism will use only one codon per amino acid. SARS CoV-2 doesn't seem to follow that convention with respect to the codons used in the furin cleavage site[27] as it uses both bat and human codons there. Doesn't all this evidence strongly suggest that the furin cleavage site was added by humans to SARS CoV-2- not nature?

Discussion:

There is clearly plenty of evidence that strongly suggests that SARS CoV-2 resulted from genetic engineering rather than natural selection. What's also clear is that while the receptor binding site of SARS CoV-2 could have come from a swap with another bat coronavirus, the furin cleavage site could not. That's because the furin cleavage site hinders the replication of SARS CoV-2 in bats- so where did it come from, and how did humans spend enough time with this unknown species for SARS CoV-2 to become well adapted to humans?

Few papers asserting that the virus arose through natural selection address the issue of homology, i.e., that RaTG13 and and SARS CoV-2 are more closely linked in evolutionary time than SARS CoV-1 and its putative bat coronavirus progenitor. The rapid adaptation to humans coupled with inconsistencies in codons in a remarkable addition, the furin cleavage site, also suggest laboratory engineering. N.B. The addition of this furin cleavage site would most certainly be considered gain of function research. The unusual and complex mechanism of SARS CoV-2 though, should make any objective scientist wonder about the origin of the virus, rather than blithely asserting that it arose through natural processes.

There are two really important questions that are being skirted here:

1) Where did the furin cleavage site come from as nothing with the same amino acid sequence has been seen in other coronaviruses?
2) How did two unusual events, the addition of a receptor binding domain well suited to humans and the addition of a furin cleavage site happen so quickly? Look at how long it took MERS to evolve from a bat coronavirus. By comparison, the transition from the bat coronavirus RaTG13 to SARS CoV-2 happened in the "blink of an eye".

Any sound scientific hypothesis can be disproven. What would it take to show that SARS CoV-2 arose naturally, rather than from genetic engineering? Simple: find another coronavirus that has a similar furin cleavage site to SARS CoV-2 and show that the animal that is host to the virus now was originally infected by RaTG13 or a very similar bat coronavirus. Given how extensively

[26] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8689951/
[27] www.pnas.org/doi/epdf/10.1073/pnas.2215826119

there's been a search for this putative intermediate species, it seems unlikely that this evidence is going to show up.

Now is the time to grapple with the possibility that the next pandemic will be caused by a genetically engineered virus. As the technology evolves, it's going to be easier to produce viruses tailored to produce infections in humans which means that more laboratories will be able to accomplish the task. That it's already so difficult to distinguish between a natural origin of SARS CoV-2 or a laboratory origin shows that genetic engineers can now make viruses that are difficult to distinguish from their natural counterparts. Previously, it was straightforward to identify a genetically modified virus as there were telltale fingerprints in the altered genetic code. No longer, although it looks like someone got a little sloppy with SARS CoV-2 based on the mixed bat/human codons[28]. It's a similar problem that now bedevils image analysis. With careful examination, it used to be easy to identify a photograph as having been manipulated, but with today's technology, image generation technology is so powerful that whether the photograph depicts reality or has been manipulated is impossible to tell. We have a similar problem with viruses. SARS CoV-2 has shown that we are unable to determine whether or not a virus has a natural origin and in fact, it seems increasingly likely that the virus was engineered. What this means is that we must prepare for future pandemics started by engineered viruses- the genie is out of the bottle.

Conclusions:

Suggestions have been made that knowing the origin of the virus is impossible and that we should move on. This idea is fallacious in two ways. First, as any good scientist knows, an experiment can be replicated. Perhaps it is time to try and replicate SARS CoV-2 in a laboratory (with appropriate safety measures!) using humanized mice with an ACE-2 receptor as the needed intermediate species? If the results, starting with RaTG13 and changing the receptor binding domain and adding a furin cleavage site look very similar to SARS CoV-2, that's a pretty good answer. A control experiment could be to use the same mice but do no genetic modifications of RaTG13 other than the receptor binding domain since that could have a natural origin. The results of this experiment should answer the question as to whether or not the furin cleavage site was added in a lab, or through some novel natural process.

The second issue with moving on involves pandemic preparedness. A great deal has been written about the need for increased surveillance to improve our response to the next pandemic. Unfortunately, most of this surveillance has been aimed at zoonotic viral origins. While zoonotic spillovers do happen on occasion (see avian flu, swine flu, etc.) if the virus does not develop the capability of human-to-human transmission, the pandemic threat from these

[28] What this shows is that SARS CoV-2 was never intended to be a bioweapon. It was created for laboratory studies, and as such, had no need for consistently using only bat codons. Including a human codon in the furin cleavage site had no deleterious effects but is a pretty clear indication of genetic engineering. Had SARS CoV-2 been intended as a bioweapon, odds are the researchers would have been more careful and only used bat codons.

spillovers is limited. If SARS CoV-2 was genetically engineered, this lab accident has killed more than 20 million people globally. Laboratories capable of these feats of genetic engineering are quickly multiplying and yet have far fewer safeguards than our nuclear arsenals which have killed two orders of magnitude fewer people.

We also have to face an additional challenge with engineered viruses compared to zoonotic viruses: novel mechanisms of action. With zoonotic viruses, our knowledge base includes a decent understanding of their mechanism of action: how they infect cells, how they replicate, and how they are released. With engineered viruses, we may be faced with new mechanisms of action which means that previously successful control strategies may not work well-potentially including vaccines. SARS CoV-2 has clearly shown that our lack of understanding that the virus had a novel mechanism of entry into cells greatly slowed the development of useful antiviral drugs and caused us to underestimate how the virus could rapidly improve its human to human transmission.

The clear lesson from SARS CoV-2 is that there needs to be far greater scrutiny of gain of function research, and we need to use not only vaccines, but other methods to decrease the severity of pandemics.