Chairman Rand Paul, M.D.

U.S. SENATE COMMITTEE ON HOMELAND SECURITY & GOVERNMENT AFFAIRS

NEWLY RELEASED DOCUMENTS SHOW PROXIMAL ORIGIN AUTHORS PRIVATELY DOUBTED THEIR OWN PAPER AND GRAPPLED WITH DEFUSE PROPOSAL

What is the Proximal Origin Paper?

The paper "The Proximal Origin of SARS-CoV-2" was published in *Nature Medicine* on March 17, 2020, seeking to clarify the origins of the ongoing pandemic. That paper, now one of the most cited academic papers in history, asserted that "any type of lab-based scenario" wasn't "plausible."

ACKNOWLEDGED AUTHORS: Kristian Andersen, Andrew Rambaut, Ian Lipkin, Eddie Holmes, Bob Garry
UNACKNOWLEDGED AUTHORS: Anthony Fauci, Francis Collins, Jeremy Farrar

The Proximal Origin authors privately put the odds of a lab origin as high as 30%, months after telling the public it wasn't plausible.

In June 2020, Andersen and Holmes closed a discussion by exchanging confidence intervals for a bat versus lab origin. **Andersen assessed 70/30 bat/lab. Holmes put it at 80/20, then 90/10.** These were private assessments, exchanged months after the paper told the public a lab origin was not plausible.

Kristian Andersen walked back the paper's central conclusion and conceded the paper's arguments were failing.

In June 2020, Andersen wrote: **"Our paper was pretty strong in saying 'there's no way', but I have less confidence in that statement at this stage."** The paper argued manipulation was implausible, but after German scientists built a reverse-genetics system for SARS-CoV-2 in under two weeks, Andersen wrote **"clearly it's not that hard."** He also admitted the paper misstated a fact about the bat-coronavirus culturing at the center of the origins question: **"We describe this in the paper and say that this work is performed 'all of the world', but that isn't true – it has exclusively been going on at WIV."**

What is the DARPA DEFUSE Proposal?

DEFUSE was a 2018 grant proposal, submitted by Peter Daszak of EcoHealth Alliance with Zhengli Shi of the Wuhan Institute of Virology and Ralph Baric of University of North Carolina, that proposed inserting furin cleavage sites into SARS-related bat coronaviruses. The furin cleavage site is a feature in SARS-CoV-2's spike protein that enhances how efficiently the virus enters human cells, and its presence became one of the central reasons many scientists suspected the virus had been engineered. In September 2021, the DEFUSE proposal was made public after a whistleblower complaint was leaked.

DEFUSE TEAM: Peter Daszak, Ralph Baric, Zhengli Shi, Linfa Wang, Tonie Rocke, Jerome Unidad

The leaked DARPA DEFUSE proposal triggered alarm among the Proximal Origin authors.

When the proposal surfaced, its overlap with the defining characteristic of SARS-CoV-2 was not lost on the Proximal Origin authors. Andrew Rambaut wrote: **"it really isn't beyond plausibility that someone went ahead and tried some of this stuff. We just need to re-convince ourselves it is coincidence."** Andersen replied: **"that's the problem. There's a big difference between 'the lab was working on bat CoVs' to 'the lab was modifying bat CoVs using features we see being unique to SC2.'"**

Faced with the DEFUSE problem, the authors quickly took actions to coverup the coverup.

As they reopened their analysis, the authors discussed the discoverability of their own communications. Rambaut: "And stay off email," then, moments later, **"Or carefully curate some emails for the FOI records."** Rambaut also proposed a call to discuss "what our strategy should be so we don't get associated with these fuckers," referring to what he called **"the Baric/Daszak shitshow."** Andersen agreed, adding: **"I also need to get something back to Tony's team at NIH."**

[Robert Garry]
I like weird = just ask @Kristian Andersen I'm a big ferret fan to, if we're considering non-bats.

[2020-05-22 16:59:31]
[Eddie Holmes]
Ferret-badger would be great.

[2020-05-22 17:30:25]
[Eddie Holmes]
According to this, one of those Imperial College reports has an Altmetric of >65,000. I find this very hard to believe, and it's not a paper. Perhaps it solves the mystery though? https://www.scienceopen.com/search#('order'~0_'context'~('collection'~('id'~'d6ba10ea-809d-4f28-96b9-d2ed475ec319'_'kind'~0)_'kind'~11)_'v'~3_'kind'~77)

[2020-05-22 17:43:48]
[Robert Garry]
Guess I missed the hoopla. I think this is an error as well - if you look at the distribution of twitterers is all in the US. If not, apparently, one of us needs to find a married blond socialist "side-piece."

[2020-05-22 17:44:31]
[Robert Garry]
There is an obvious candidate BTW.

[2020-05-22 18:18:41]
[Robert Garry]
It's time for Ian to step up.

[2020-05-22 18:23:17]
[Eddie Holmes]
Yes, I think it's an error. Butt lesion has been very quiet lately.

[2020-05-23 14:24:13]
[Eddie Holmes]
Just a had a thought: for the GVP to work they would have to sequence gazillions of viruses. Then, to work out which of these might infect humans, they would then have to test them all in a range of human cells. We previously pointed out the crazy enormity of this task. But, it's not just size: what they are effectively proposed are 100,000s of GoF experiments for viruses that could potentially infect humans. Even if we don't think the virus came out of a lab, surely this is now an unacceptable risk?

[2020-05-23 14:31:01]
[Robert Garry]
That's a very good point Eddie - needs to see the light of day.

[2020-05-23 14:31:48]
[Eddie Holmes]
Not quite sure how to say it without encouraging the lab escape crowd for the current outbreak?

[2020-05-23 14:32:39]
[Kristian Andersen]
Exactly - that’s why I haven’t said anything :wink:

[2020-05-23 14:34:19]
[Eddie Holmes]
Your ahead of me Kristian. I think more about how this can be done.

[2020-05-23 14:37:02]
[Kristian Andersen]

[Kristian Andersen]
While I knew about the furin site insertion experiments, I did know realize this (that the insertion in SARS2 is exactly where they inserted it experimentally in SARS):

https://twitter.com/Ayjchan/status/1266805310313967617?s=20

[2020-06-11 13:35:58]
[Kristian Andersen]
Gotta say - some smoking(ish) guns starting to appear that I'm not comfortable with and Alina here is touching on a lot of points we have discussed previously that were concerning (e.g., lack of selection - I should have looked into that a little closer earlier :wink:). The furin site in SARS2 is exactly the same as it is in some of the HKU1 sequences.

When I talked to Farzan way back in the day he mentioned that the one thing he'd be looking for was whether SARS2 would lose the furin site under different conditions - we now know this to be true (primarily in T/C, but there are examples in people too - intrahost).

[2020-06-11 13:58:46]
[Robert Garry]
"exactly where they inserted it experimentally in SARS" Sure - but that site with the single R is a required cleavage site - if you're going to convert to a furin site then adding RRA at that exact position is the only thing that makes sense - or even RRAR to make it a super-optimal site like Nunberg did - what would not make sense is to stick a proline in and then to do the whole insertion out-of-frame.

[2020-06-11 14:08:35]
[Kristian Andersen]
Yeah, agreed - however, this exact site already exist in other CoVs (just not in exactly this site) - not in HKU1 as I said, but in that Feline CoV.

The part I'm really struggling with is that at the end of the day, we really don't have any hard evidence one way or the other - and especially given some of the recent evidence, we also can't rule out that somebody actually put it in there. That's obviously not to say that somebody _*did*_, but we can't rule it out. Our paper was pretty strong in saying "there's no way", but I have less confidence in that statement at this stage.

[2020-06-11 14:33:23]
[Eddie Holmes]
What smoking guns Kristian? I don't quite see it. What recent evidence are you talking about? That Follis paper was literally the first one I looked at. Doesn't it's presence in HKU1 suggest that these events in nature a lot? Do we know what animal HKU1 comes from? I was reading Mike's paper to suggest that D614G was under selection to increase the infectivity of SARS-CoV-2, because it was directly related to the furin cleavage insertion, such that it not perfectly adapted to humans on emergence.

[2020-06-11 14:33:34]
[Eddie Holmes]
What about Bill Gallaher's evidence?

[2020-06-11 14:34:14]
[Robert Garry]
HKU1 actually does have an optimal furin site - some strains have a insert or deletion of SSS - yes some of these S have predicted O-glycans [shared file(s): HKU-1 Spike.pdf]

[2020-06-11 14:38:27]
[Robert Garry]
"animal HKU1 comes from" Rodents maybe - probably

[2020-06-11 14:44:30]
[Eddie Holmes]

May be. Perhaps rodents are involved in SARS-CoV-2? I'm really struggling to understand why someone would "put" a furin site into a random bat virus they had not previously sequenced and published. Has the Baric group ever done such an experiment? Accidental lab escape is one thing, but why would deliberately do this to a bat virus? Then why would publish the sequence of a related bat virus that puts you in frame? There was no need to that if you were guilty.

[2020-06-11 15:02:34]

[Robert Garry]

"Then why would publish the sequence of a related bat virus that puts you in frame?" To throw off M15 and Pompeo?

[2020-06-11 15:13:08]

[Kristian Andersen]

People have put furin sites into CoVs previously - here's one example from SARS: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7111780/

[2020-06-11 15:13:50]

[Kristian Andersen]

There's a paper where they put it into a bat virus and shows it has much broader host range - I believe from Baric's group. I'll find it.

[2020-06-11 15:15:01]

[Eddie Holmes]

At S1/S2?

[2020-06-11 15:15:46]

[Kristian Andersen]

Yup - the paper above is _exactly_ where it was inserted in SARS2 - that's the part I missed previously (I have been aware of these papers all the time).

[2020-06-11 15:16:22]

[Kristian Andersen]

(BUT, the inserted site _is not_ the same as in the paper - that would have been a dead giveaway)

[2020-06-11 15:16:32]

[Eddie Holmes]

We're going round in circles...that's the Follis paper...the first paper we discussed and I sent you in early Feb.

[2020-06-11 15:18:29]

[Eddie Holmes]

My take is that furin cleavage sites are *everywhere* in natural viruses e.g. MERS. I'm revising a paper on avian avulaviruses and they have one. To me, there is a strong natural prior.

[2020-06-11 15:18:52]

[Kristian Andersen]

Yes - I have been aware of the Follis paper all along - I was _not_ aware that their insertion was exactly where it is in SARS2

[2020-06-11 15:20:07]

[Eddie Holmes]

Also, the Zhou et al. paper never call it a furin cleavage site insertion and I think the alignment in that paper is the optimal one. If so, it is not a simple 'shift' but rather a complex set of indel events.

[2020-06-11 15:20:46]

[Kristian Andersen]

Here's the paper from Baric - not the insertion, but showing that cleavage broadens host range (https://pubmed.ncbi.nlm.nih.gov/31801868/) - which is what furin does. This is also not a new paper - I have been aware of this all along as well, so not a smoking gun, but there are several concerns here related to tissue culture

[2020-06-11 15:21:34]

[Kristian Andersen]

I don't think the Zhou et al. paper shows that this is multiple indels - to me it just looks like a region not aligning very well?

[2020-06-11 15:22:17]

[Kristian Andersen]

Let me spend some time on a table with things that bothers me - because there are several.

[2020-06-11 15:23:20]

[Eddie Holmes]

That would be useful. Thanks.

[2020-06-11 15:26:20]

[Kristian Andersen]

Yeah, let me get it all down - including the silly stuff :wink:

[2020-06-11 15:42:57]

[Eddie Holmes]

Make sure there is a column in your table that can be used for 'possible natural explanation'. For example, I don't see why adaptation in animals/culture would then produce a virus that would need not to adapt in humans themselves.

[2020-06-11 16:18:10]

[Eddie Holmes]

I also think that focusing on these genetic details - which are not conclusive - is in danger of missing the bigger picture: that bats (and like other wildlife) carry a huge diversity of coronaviruses some of which are closely related to SARS-CoV-2; that coronaviruses link species boundaries all the time; that 4 other CoVs have emerged in humans in the last 20 years; that furin cleavage sites are common in viruses and come and go all the time; that people have been warning about emerging CoVs for years. This is not a blue sky event. It just seems to me that we are going over the same arguments we went through in Feb.

[2020-06-11 16:42:42]

[Kristian Andersen]

I of course agree with all of this (and still believe this is a natural virus), but the issue is that some of the new evidence certainly is peculiar - plus some additional details that have come to light, e.g., acquisition of furin site in tissue culture in bovine CoV.

Nothing has changed in terms of prior risk of lab accident, but the issue is that we're dealing with the fact that WIV has been doing culturing of bat CoVs for over a decade exactly to understand what it takes for these viruses to emerge in humans (in BSL-2). We describe this in the paper and say that this work is performed "all of the world", but that isn't true - it has exclusively been going on at WIV. The EgoHealth grant was also specifically funded for this type of work (and described the kind of experiments to be done in tissue culture). This research primarily involves collecting and culturing viruses from bats in Yunnan - which is where RaTG13 is from and it seems plausible that SARS2 is also from there (no roosting bats in Wuhan in winter). A lot of the GOF work on SARS-like CoVs is also from WIV, although there are also other labs that have been doing that (e.g., Baric).

None of this tells us anything new - but it means that given no data, I find both scenarios to be equally probable. Given the data we had seen at the time of our writing, I was highly skewed towards 'natural'. But with some of this new data (plus stuff I didn't know previously), I'm moving much more towards the middle (again).

Suffice to say, since writing the paper I have explained to a ton of scientists (and policy makers) over and over again why this is a natural virus, and I have been pretty surprised to learn that most scientists I talk to believe there is a likely connection to the lab - people that are themselves doing GOF and culture-type of experiments, they know the

risks and don't seem to think that a natural explanation is more likely than lab culture.

[2020-06-11 17:01:47]

[Eddie Holmes]

Fair points. But that work isn't exclusively done at the WIV because as you say yourself the Baric lab are doing it. I still don't quite see what the new data is. The bovine stuff? What else is there? To me, there is more evidence against lab escape than when we wrote the paper. I think the bats not roosting in winter argument is nothing because I reckon it went through an intermediate host anyway. Do you have a copy of the EgoHealth grant?

[2020-06-11 17:07:00]

[Kristian Andersen]

Aim3: https://projectreporter.nih.gov/project_info_description.cfm?aid=9819304&icde=50404048&ddparam=&ddvalue=&ddsub=&cr=1&csb=FY&cs=DESC&pball=

[2020-06-11 17:11:58]

[Kristian Andersen]

> But that work isn't exclusively done at the WIV because as you say yourself the Baric lab are doing it
No, that work _is_ exclusively at WIV AFAIK. Baric's lab is doing (did) gain of function research where they created reverse genetics systems to study the viruses. All of this is done in BSL-3, so much (much!) less risk of exposure. The work in Shi's lab is different - here they are taking bat CoVs and culturing them under BSL-2 in cell lines from different animals - to e.g., understand which ones can infect human cells and what type of mutations that occur in T/C may make them permissive. They probably have more than 10 papers where they describe that type of work on all kinds of SARS-like CoVs.

[2020-06-11 17:14:11]

[Eddie Holmes]

Got it, thanks. That helps.

[2020-06-11 17:16:59]

[Eddie Holmes]

Have the Shi lab published all the viruses they use in these culture experiments? Do they find the same mutations that appear in SARS-CoV-2?

[2020-06-11 17:21:19]

[Kristian Andersen]

In our paper we argue against (a) manipulation and (b) tissue culture. Our main arguments were that manipulation is hard, but the German's came out and created a reverse genetics system of SARS2 in less than two weeks, so clearly it's not that hard. Our arguments against tissue culture mostly came down to the O-linked glycans and a 'mucin-like' domain. But by now it seems clear that these don't actually form a mucin-like domain, but rather those glycans modulate the function of the furin site. All our arguments about natural viruses emerging, the RBD identical in pangolin CoVs, etc., all still stand.

The main new things that bothers me the most:
1. Lack of selection (a lot of the selection is for replication in a particular host species, so yes, I would assume culture would lover selection during subsequent transmission in humans - however, immune selection will still occur).
2. Gain of furin cleavage of bovine CoV in tissue culture.
3. Loss of furin site in tissue culture meaning it's active.
4. The little details about the furin site - e.g., that it's exactly in the spot where experiments had previously inserted it, the fact that the exact sequence exist in a cat CoV, Shi herself being sketchy with details and considering lab accident, etc. But all of this can go both ways

[2020-06-11 17:21:58]

[Kristian Andersen]

Unclear to me if they published sequences of all these viruses - it's a lot and names are changing over time (e.g., RaTG13 had a different name previously).

[2020-06-11 17:23:02]

[Kristian Andersen]
And yes - of the six key mutations in the RBD, one of them occur in tissue culture for SARS (*but* it's also present in the pangolin RBD).

[2020-06-11 17:27:24]
[Eddie Holmes]
Are you saying they deliberately inserted the furin cleavage site? I see no evidence of them ever doing that before. (I don't think RaTG13 had a different name...I think there a partial sequence from another virus that is identical. Can we do a quick zoom?

[2020-06-11 17:38:22]
[Eddie Holmes]
To me, "Loss of furin site in tissue culture meaning it's active" strongly argues AGAINST culturing as being the cause of the cleavage site insertion.

[2020-06-11 17:44:39]
[Kristian Andersen]
Just making some dinner - let's Zoom in ~30mins?

[2020-06-11 17:47:04]
[Eddie Holmes]
Let's say 6:30 pm time. OK?

[2020-06-11 18:29:24]
[Eddie Holmes]
https://uni-sydney.zoom.us/j/98148315438

[2020-06-11 18:30:12]
[Kristian Andersen]
I'm on!

[2020-06-11 20:14:23]
[Kristian Andersen]
:bat:/■
Eddie: 80/20
Kristian: 70/30

[2020-06-11 20:34:35]
[Eddie Holmes]
90/10. I think.

[2020-06-11 20:37:51]
[Eddie Holmes]
Strictly confidentially, I literally (2 mins ago) just got an email that says: "Recently, we isolated a pangolin-Cov that is highly related with SARS-CoV-2 (https://www.nature.com/articles/s41586-020-2313-x). We further found that the infected pangolins showed similar CT scans with COVID-19 patients (please see the attachment, a and b are virus-positive pangolins, while c is virus-negative). Thus, we further did the transcriptome of their multiple tissue, and found they showed similar immune response as that of COVID-19 patients, that is, an inadequate interferon response, but excessive inflammatory response and an over-exuberant cytokine response.In addition, we found other interesting results". I don't the viruses are any closer to the ones we have but the clinical/transcriptome results are interesting...if true (you can never tell) would you expect a clinically similar infection in an animal in nature if this had come out of a lab?

[2020-06-11 20:38:25]
[Eddie Holmes]
I will of course agree to help to see what more I can discover.

MHV changes its FCS all the time

[2021-09-14 07:32:05]

[Robert Garry]

See here - no making fun of my tree. [shared file(s): image.png]

[2021-09-21 00:40:05]

[Eddie Holmes]

Got another FOIA from [redacted]. This will be declined again and probably end up in court again. This is what my Uni told me (apparently the Uni cyber security people have kindly collated all my emails for me):

[2021-09-21 00:40:09]

[Eddie Holmes]

And for context, the application was another from [redacted], Associated Press.

The final scope of the application is:
All University of Sydney email correspondence between personnel of the University's Viral Evolution Lab and:
· Anthony Fauci (email address afauci@niaid.nih.gov, AF10r@nih.gov)
_· Kristian Anderson (email address [redacted])
· Marion Koopmans (email address [redacted])
· Andrew Rambaut (email address [redacted])
· Jeremy Farrar (email address [redacted])
· Collins Francis (email address francis.collins@nih.gov)
containing the search terms "Phytogenic", "coronavirus", "Wuhan", and "GISAID" for the period 15 December 2019 to 15 June 2020.

[2021-09-21 00:41:43]

[Andrew Rambaut]

I can confidently predict that the number of emails containing the words - "Phytogenic", "coronavirus", "Wuhan", _and_ "GISAID" is zero.

[2021-09-21 00:43:46]

[Andrew Rambaut]

Ignoring the spelling of 'phytogenic' this is amateur stuff - what useless keywords.

[2021-09-21 00:48:18]

[Eddie Holmes]

Apparently my Uni people corrected 'phytogenic' to 'phylogenetic'

[2021-09-21 00:48:47]

[Andrew Rambaut]

When they say 'and' do they actually mean 'or'?

[2021-09-21 00:49:10]

[Eddie Holmes]

I guess or.

[2021-09-21 07:58:35]

[Andrew Rambaut]

Going back to this channel for this but this stuff about the DARPA proposal makes me even more angry about the DT letter (if it is indeed Baric) - he knew how suspicious a FCS would look back then.

[2021-09-21 08:00:18]

[Kristian Andersen]

And his attacks on me for even suggesting we needed to take a look at this. He's a two-faced bastard, that's for sure.

[2021-09-21 08:01:59]

[Kristian Andersen]

IMO we really _do_ need to take a close look at what's described in this grant and look back at our (now expanded) alignments to see if anything pops up. E.g., Bob mentioned that there are differences in Laos bat vs SC2 when it comes to N-linked glycans, which isn't particularly reassuring - that's the kind of work described on page 11.

[2021-09-21 08:05:12]

[Andrew Rambaut]

I agree - need to stay on top of this.

[2021-09-21 08:05:42]

[Kristian Andersen]

Yes - this can't (shouldn't) be dismissed out of hand.

[2021-09-21 08:06:07]

[Andrew Rambaut]

If there is anything suspicious - better that we say it and then address it. If there isn't then we can let it blow over.

[2021-09-21 08:06:40]

[Kristian Andersen]

Exactly. I suspect it'll be the latter.

[2021-09-21 08:08:52]

[Andrew Rambaut]

Me too. But it really isn't beyond plausibility that someone went ahead and tried some of this stuff. We just need to re-convince ourselves it is coincidence.

[2021-09-21 08:10:14]

[Kristian Andersen]

Correct - that's the problem. There's a big difference between "the lab was working on bat CoVs" to "the lab was modifying bat CoVs using features we see being unique to SC2".

[2021-09-21 08:10:33]

[Andrew Rambaut]

Key question is - would anyone chose to put the FCS we see in SC2 in and put it out of frame.

[2021-09-21 08:11:21]

[Kristian Andersen]

Would be a very strange way to do it, but not impossible - depending on their cloning strategy.

[2021-09-21 08:12:04]

[Andrew Rambaut]

If you wanted to insert an FCS you would pick the AAs you wanted, pick the codons you wanted and then insert it.

[2021-09-21 08:12:46]

[Andrew Rambaut]

The alternative is that it was inserted in frame and then mutated to make it look out of frame

[2021-09-21 08:14:19]

[Kristian Andersen]

Again, it depends on cloning strategy - if the use restriction enzymes and there would be an easy way to insert an FCS with PCR utilizing REs already present in the sequence, then it could be a way to do it. Not the optimal way, for sure, but sometimes ease trumps perfection.

[2021-09-21 08:16:32]

[Andrew Rambaut]
Would you pick CGG for example? Would you consider what codon to use for an R or just pick one arbitrarily.

[2021-09-21 08:21:23]
[Kristian Andersen]
CGG would be an unusual choice IMO - not a ‘optimized’ R and sorta middle-of-the-pack as it comes to usage in human genomes. Very rare, of course, in viruses because it’s a CpG.

[2021-09-21 08:24:03]
[Andrew Rambaut]
And you have 6 codons to chose from.

[2021-09-21 08:25:24]
[Kristian Andersen]
Correct. Key here too is that if you were to ‘optimize’ the FCS sequence (as is commonly done for these types of experiments) you would _not_ end up with the FCS sequence we see, including the CGG codons. But again, it doesn’t really tell us much.

[2021-09-21 08:26:15]
[Kristian Andersen]
What I think we have to look _very_ closely at are all the specific sites they talk about in the grant and then compare across our alignments - is SC2 unique in those positions compared to, say, the BANAL viruses? Does it flip back to SARS1 at some of these positions, etc.

[2021-09-21 08:26:48]
[Andrew Rambaut]
Yup.

[2021-09-21 08:27:39]
[Kristian Andersen]
I’ll get some of this started later today - it’s important to get the insights irrespective of what it’s going to end up showing.

[2021-09-21 08:28:55]
[Andrew Rambaut]
Would there be any reason to pick the SARS2 backbone for doing these? Assuming they had the genome sequence way back at the start?

[2021-09-21 08:30:30]
[Kristian Andersen]
Well, in the grant they specifically talk about *new* viruses - so a SC2 precursor could have been one of those “low risk” viruses they talk about and then they started fiddling with it.

[2021-09-21 08:34:27]
[Andrew Rambaut]
They would have selected it from candidates by genome sequencing it. I guess they would have seen an ACE-2 binding RBD and thought that is the one to try an FCS in?

[2021-09-21 08:37:08]
[Andrew Rambaut]
Or they just were selecting viruses by seeing if they could get any to culture and then worry about genome sequencing?

[2021-09-21 08:48:58]
[Kristian Andersen]
The former - which is the approach they describe in the grant.

[2021-09-21 08:49:44]

[Kristian Andersen]

Culture is very challenging so I don't think that approach makes sense.

[2021-09-21 09:00:15]

[Kristian Andersen]

Here's what I suggest we do:

• Make (spike) alignments with SC1, SARSr-CoVs, RaTG13(+close friends), Pangos, BANAL, SHC014, rs4237, SC2
• Mark 'key' sites based on what's in the grant - @Robert Garry, can you please help highlight the N-linked glycans based on Wuhan Hu-1 coordinates
• Check to see if there's anything odd between SC2, basal viruses, and SC1
• Look at e.g., codon usage at those sites if anything stands out
• Cross-check with how those sites have evolved during the pandemic

[2021-09-21 09:01:22]

[Andrew Rambaut]

And stay off email

[2021-09-21 09:02:00]

[Andrew Rambaut]

Or carefully curate some emails for the FOI records

[2021-09-21 09:07:39]

[Robert Garry]

OK I'm on my bit...

[2021-09-21 09:07:43]

[Kristian Andersen]

Exactly - let's dig around a little and then start an email chain.

I'm in Zoom calls most of the day, but I'll dive in in the afternoon.

[2021-09-21 09:09:52]

[Kristian Andersen]

Actually, let me get started - I just cleared my calendar for the next hour.

[2021-09-21 09:34:20]

[Robert Garry]

Is this a good set for the SC1 related viruses - or others (some more civet ones)? [shared file(s): image.png]

[2021-09-21 09:35:11]

[Kristian Andersen]

I'm getting (and annotating) the genomes now

[2021-09-21 09:37:43]

[Andrew Rambaut]

Ooh.. Can't wait.

[2021-09-21 09:39:31]

[Andrew Rambaut]

Probably worth having the full genomes aligned even if we only focus on the close relatives in the spike

[2021-09-21 09:41:18]

[Kristian Andersen]

Yup - I'm getting all the full length genomes first and then we can cut alignments out from there. Getting them annotated too - future Kristian will thank present Kristian.

[2021-09-21 11:09:02]

[Kristian Andersen]

Alright, first attempt at an annotated alignment with relevant sequences. Am I missing anything? Any other sequences mentioned in the grant I should include?

@Robert Garry, once I have the coordinates of the N-linked glycs I'll include those in the alignment as well. Once we have all of those annotated, I'll create Spike alignments, including AA and NT versions. [shared file(s): 2021.09.21_alignment-full.geneious]

[2021-09-21 11:45:41]

[Robert Garry]

excellent!

[2021-09-21 13:59:31]

[Eddie Holmes]

What also annoys me is that Peter should have seen this coming and it is worse than he was suggesting a few days ago. He's a jackass. We still have no evidence for SC2 in that lab and they must have sequenced it first. But it is very striking that a bat-derived CoV with an FCS turns up in a city that was on a grant proposing to muck around with FCSs in bat-derived CoVs.

[2021-09-21 14:00:43]

[Eddie Holmes]

I might see if I can talk to Linfa about it.

[2021-09-21 15:25:59]

[Kristian Andersen]

Getting Linfa's take on this would be great - he's on the grant too and he also hasn't mentioned anything about this.

[2021-09-21 15:27:37]

[Eddie Holmes]

I'll get onto it. I'm also going to have to chat to the intelligence folk.

[2021-09-21 16:02:14]

[Robert Garry]

Some of the most valuable info is going to come from sorting out the parallels btwn SC1 and SC2. It's ironic that two KEY pieces surfaced in the past few weeks- the hubel civet viruses and the BANAL bat viruses. With SC1 and a little elbow grease you'll see the adaptions from bat to civet to human and then human to human. With SC2 we'll have all the same genomes except the intermediate host (and I think a good assumption that there is one). In both SC1 and SC2 you've got highly similar N-glycan changes in the NTD followed by very similar and specific RBD changes. It's possible the NTD changes opened the door for the RBD changes. Put the FCS aside - nobody did these previously undiagnosed NTD and RBD changes by design.

[2021-09-21 16:05:51]

[Kristian Andersen]

Yup, all important points Bob. As for the glycans - do you have the coordinates?

[2021-09-21 16:06:11]

[Kristian Andersen]

I'm done with my Zoom calls so I'll focus on this for the rest of the day/night.

[2021-09-21 16:24:57]

[Robert Garry]

For SC2 to laos bat 52 its N 30, 74 and 370.

[2021-09-21 16:25:21]
[Robert Garry]
SC1 coming

[2021-09-21 16:37:56]
[Eddie Holmes]
I'd love to know what a 'human specific furin cleavage site' is.

[2021-09-21 16:48:28]
[Kristian Andersen]
It's not "PRRAR", that's for sure.

[2021-09-21 16:48:58]
[Kristian Andersen]
@Robert Garry - I'm using NetNGlyc and since N-linked glycans are easy to predict I think I have what I need

[2021-09-21 16:51:41]
[Robert Garry]
for sc1 the ones I have my eye on are N 29, 73 and maybe 227

[2021-09-21 16:52:49]
[Robert Garry]
variable with close bat viruses like KY417150

[2021-09-21 17:14:25]
[Kristian Andersen]
@Robert Garry what are the key N-linked sites for the DC-SIGN/L-SIGN stuff? I have my eye focused on residue 370 in SC2 (357 in SC1) since that is a FULLY conserved N-linked glycan across _all_ sarbecos, _except_ in SC2 because residue 372 changes from a fully conserved T to an A (via a single A>G transition in the first codon position).

In the RBD there are three N-linked glycans in SC2/BANAL, so two are conserved, one is not.

[2021-09-21 17:19:19]
[Kristian Andersen]
We have _some_ information about what this particular residue does in SC2: https://pubmed.ncbi.nlm.nih.gov/34289344/

[2021-09-21 18:24:13]
[Eddie Holmes]
MHV has RRAR. FCoV has PRRAR. Would they have used the FCS's from these (although different codons)? I note that these viruses been used by groups in Wuhan in a different context:

[2021-09-21 18:24:44]
[Eddie Holmes]
shared file(s): JVI.00948-15.pdf

[2021-09-22 14:05:51]
[Andrew Rambaut]
I think we that maybe we could have a chat tomorrow about the Baric/Daszak shitshow? I would just like to discuss what our strategy should be so we don't get associated with these fuckers.

[2021-09-22 14:11:56]
[Kristian Andersen]
Yeah, I think that would be good - we can't ignore this and I also need to get something back to Tony's team at NIH.

[2021-09-22 14:13:05]
[Kristian Andersen]
Too early Sydney time? [shared file(s): Screen Shot 2021-09-22 at 14.12.46.png]

[2021-09-22 14:13:22]
[Andrew Rambaut]
I was just starting to have fun again looking at Laotian bat viruses…

[2021-09-22 14:13:45]
[Andrew Rambaut]
I can do later if needed.

[2021-09-22 14:15:02]
[Kristian Andersen]
Yeah, me too - and I think those viruses are incredibly important.

How about tomorrow half an hour later?

[2021-09-22 14:15:57]
[Andrew Rambaut]
Good for me. Will let Eddie say what suits him.

[2021-09-22 14:35:58]
[Kristian Andersen]
@Eddie Holmes @Robert Garry - would tomorrow 7am/2pm/4pm/9:30pm work for you?

[2021-09-22 14:39:17]
[Eddie Holmes]
Yes, I can probably make 7 am. 10 pm Edinburgh I think?

[2021-09-22 14:41:13]
[Robert Garry]
just let me know when central time

[2021-09-22 14:41:38]
[Kristian Andersen]
Yeah, 10pm Edinburgh - 4pm central :wink:

[2021-09-22 14:41:46]
[Kristian Andersen]
I'll email a Zoom invite.

[2021-09-22 14:42:48]
[Kristian Andersen]
Emailed invites.

[2021-09-22 14:51:14]
[Robert Garry]
all good

[2021-09-22 14:54:24]
[Eddie Holmes]
Great meeting title

[2021-09-23 12:35:38]

[Kristian Andersen]
Should we invite Joel and/or Mike on the call we have in 1.5hrs? We had a quick chat about the grant on another call this morning and Mike has that upcoming Science debate.

[2021-09-23 12:48:22]
[Eddie Holmes]
No

[2021-09-23 12:50:00]
[Eddie Holmes]
They can be talked to separately.

[2021-09-23 12:50:00]
[Kristian Andersen]
All good

[2021-09-23 13:10:48]
[Robert Garry]
The Dean just called me to his office to fix somebody's crisis - I told him I had 20 minutes, but possible I'll be 5 minutes late. Please start without me.

[2021-09-23 16:26:28]
[Kristian Andersen]
Alignments [shared file(s): 2021.09.21_alignment-spike-aa.geneious, 2021.09.21_alignment-spike.geneious]

[2021-09-23 16:31:22]
[Andrew Rambaut]
https://docs.house.gov/Committee/Calendar/ByEvent.aspx?EventID=112880

[2021-09-23 16:32:08]
[Andrew Rambaut]
This document: https://docs.house.gov/meetings/SY/SY21/20210714/112880/HHRG-117-SY21-Bio-RelmanD-20210714.pdf

Authors: David [shared file(s): image.png]

[2021-09-23 16:32:19]
[Andrew Rambaut]
MacOs Version 11.4

[2021-09-23 16:32:52]
[Andrew Rambaut]
From earlier this year - so probably not Relman then.

[2021-09-23 16:35:13]
[Andrew Rambaut]
Jesse’s CV https://brotmanbaty.org/wp-content/uploads/2019/12/BLOOM-JESSE-CV.pdf [shared file(s): BLOOM-JESSE-CV.pdf]

[2021-09-23 16:35:29]
[Andrew Rambaut]
Must be at least 2018

[2021-09-23 16:35:46]
[Andrew Rambaut]