ple or quadruple antibiotic therapy through oral and inhaled routes, for at least a year, depending on subsequent culture results (*13*). Initiation of an unpleasant 12-month treatment course in a setting where a substantial proportion of patients, in retrospect, do not need the therapy is difficult to justify. But the data from Bryant *et al.* suggests that there may also be a risk in waiting. If it is indeed the case that *M. abscessus* evolves within the lung to evade the immune system and antibiotics, timely treatment is more likely to result in successful eradication of the infection.

A WGS approach, using highly accurate phenotyping of new isolates of NTM, may help to risk-stratify treatment decisions, and a carefully designed randomized clinical trial of current versus more aggressive approaches to NTM infection in this setting, including early treatment decisions and newer antibiotics, is required. Efforts to inform this trial design are ongoing (NCT02419989), but barriers remain, including availability of repurposed tuberculosis drugs that target *M. abscessus* (bedaquiline and clofazimine) as well as newer agents (such as relebactam, omadacycline, and tedizolid). Whether therapies such as specific bacteriophages (viruses that target bacteria) can be used as adjuncts (*14*), or in place of antibiotics, remains to be determined.

In the 30 years since the identification of mutations in the cystic fibrosis transmembrane regulator (*CFTR*) gene on chromosome 7 as the genetic basis of CF, care has changed markedly. For the majority of patients with the health care resources to support them, new "modulator" therapies (such as elexacaftor, ivacaftor, tezacaftor) promise improved lung health and extension of life spans with CF (*15*). The data presented by Bryant *et al.* are a timely reminder that individuals with CF remain at risk of infection with this highly resistant organism, and that vigilance regarding infection control measures must remain a key focus in CF care. ■

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CORONAVIRUS

How SARS-CoV-2 first adapted in humans

An early spike protein mutation promotes transmission and will shape the next vaccines

By **Hyeryun Choe** and **Michael Farzan**

Viruses need entry proteins to penetrate the cells where they will replicate. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) version is called the spike or S protein. The S protein, also the target of the current vaccines, is quickly adapting to its new human hosts. It took its first major step in this direction early in 2020, when its amino acid 614 (of 1297) changed from an aspartic acid (D) to a glycine (G). Viruses bearing this D614G mutation transmit among humans more rapidly and now form the majority in circulation. On page 525 of this issue, Zhang *et al.* (*1*) use careful structural analyses to reveal how D614G changed the S protein to accelerate the pandemic.

Early in the pandemic, in the scramble to create tools to study SARS-CoV-2, investigators developed pseudovirus systems to measure infection in a safe, easily quantifiable way. These systems express a viral entry protein on the surface of a reporter virus used to monitor cell entry and have been used for years to study many such proteins, including the S protein of "classic" SARS-CoV-1. Frustratingly, pseudoviruses built from the SARS-CoV-2 S protein produced much lower signals than those based on the very similar SARS-CoV-1 S protein. This was perplexing because biochemical studies of SARS-CoV-1 and SARS-CoV-2 S-protein receptor binding domains (RBDs) made clear that the SARS-CoV-2 RBD bound their common receptor, angiotensin-converting enzyme 2 (ACE2), with higher affinity than that of SARS-CoV-1 (*2*, *3*). Faced with inefficient assays, many virologists landed on the same solution as their structural biology colleagues: Mutate the S-protein site that is cleaved by furin-like proteases in virus-producing cells (*2*). This change kept the S-protein S1 domain, which contains the RBD and binds ACE2, covalently linked to its S2 domain, which anchors the S protein to the virion. Notably, some—but not all—of these furin-site mutations significantly improved pseudovirus infection of cells (*4*).

This fix solved a technical problem, but it deepened a mystery. Although a number of distantly related coronaviruses carry furin cleavage sites at their S1-S2 boundaries, the SARS-CoV-1 S protein, and those of all known bat-derived viruses from the same Sarbecovirus lineage, lack this site. Instead of being cleaved in virus-producing cells, their S proteins are cleaved by different proteases while the virus is engaging ACE2 in the next, yet-to-be-infected cell (*5*). As it happened, furin-site mutations that improved SARS-CoV-2 S-protein function in pseudoviruses allowed the modified S protein to work with these later-stage enzymes, just like the SARS-CoV-1 version. Why then did the SARS-CoV-2 furin site persist, even though it made infection in cell culture less efficient? Indeed, viruses passaged in culture regularly lost this site. Does it somehow improve viral transmission? Would it eventually disappear over the course of the pandemic?

In the summer of 2020, Korber *et al.* sounded an alarm about a "mutation of concern," namely D614G (*6*). In the laboratory, this change obviated the need to eliminate the S-protein furin site, apparently correcting a design flaw associated with this unusual cleavage site (*4*, *6*). Animal studies with otherwise identical viruses showed markedly greater replication of the D614G variant in the upper respiratory tract, a site important for transmission (*7*, *8*). By contrast, no significant differences between the two viruses were seen in the lower respiratory tract, a site responsible for more severe disease (*7*). These observations are consistent with the current consensus that D614G, now present in most circulating viruses, enhances viral transmission, but unlike more recent acquired mutations in S1 [e.g., Asn^{501}→Tyr (N501Y)], it does not change the rate of hospitalizations.

The underlying mechanism for this fitness advantage remained a point of controversy. Here, a second unusual property of the S protein, in this case shared with SARS-CoV-1, became relevant. The SARS-CoV-2 S protein, like most entry proteins of viruses with a lipid membrane, assembles into trimers. Typically, during the process of virion assembly, viral entry proteins subtly change their

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conformations, but it is unusual for these proteins to break their three-fold symmetry before they bind their receptor. However, the mature SARS-CoV-2 S protein often assumes an asymmetrical arrangement whereby one of its three RBDs assumes an open or "up" conformation (*1*, *9*). Only RBDs in this up conformation can bind ACE2. Once it does so, the S1 domains dissociate from S2, and S2 undergoes a pronounced rearrangement to a "postfusion" state. The released energy of this rearrangement drives the viral and cell membranes to fuse and gives the virus access to the cell interior.

To explain D614G fitness, some investigators focused on the impact of D614G on the frequency with which this one-up conformation could be found, suggesting that more efficient engagement of the receptor accounted for the enhanced transmissibility of viruses bearing this mutation (*10*, *11*). Others noted that S proteins of D614G-expressing viruses fell apart less frequently, an effect perhaps amplified in the challenging environment of a living organism. They observed that D614G helped the S1 domain cling to S2, preventing S2 from prematurely and unproductively assuming its postfusion conformation (*4*, *9*, *12*). Thus, the virus had more functional S proteins that could bind and infect the next cell.

To cut through this controversy, Zhang *et al.* solved the structure and provided detailed analyses of both D614 and G614 S proteins in multiple states. They first noted that, as they and others had previously observed, the loss of D614 in S1 breaks an ionic bond to a proximal lysine, K854, in S2 (*9*). Loss of this salt bridge is initially counterintuitive because it would loosen the association between S1 and S2, although it might ease the movement of the RBD into the up configuration. However, the structures from Zhang *et al.* show that a major difference between S proteins with and without D614G is the visibly greater ordering in G614 S proteins of a region spanning residues 620 to 640, which the authors call the "630 loop." This loop is just downstream of G614. It is therefore possible that either the loss of the D614-K854 salt bridge, or the greater backbone flexibility that a glycine affords, helps the 630 loop nestle more tightly in a canyon formed by two larger S-protein domains (the amino-terminal domain and carboxyl-terminal domain 1). Regardless, this loop is found in a more rigid and stable arrangement between these domains when residue 614 is a G than when it is a D.

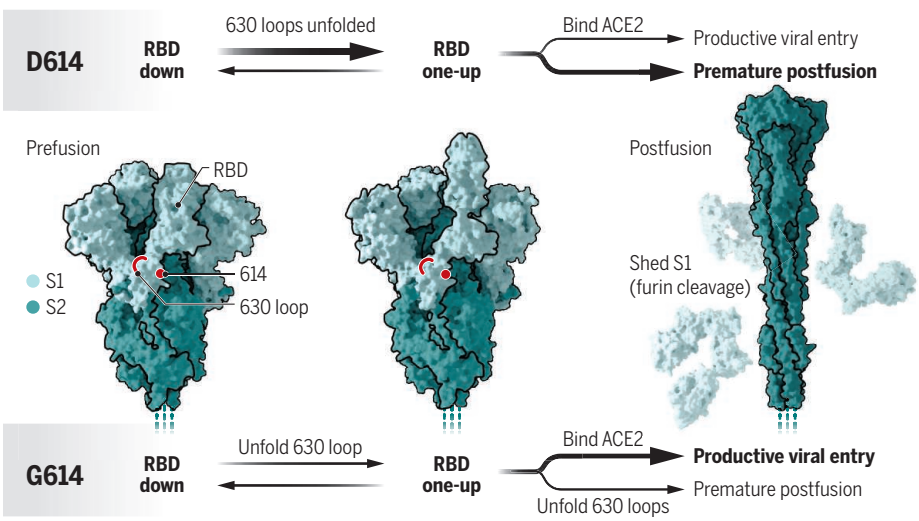
The key is that both the RBD-up conformation and dissociation of S1 from S2—enabled by furin cleavage—require disordering of the 630 loop. Thus, the RBD-up conformation can be more easily accessed with the original D614 S protein, but once this conformation is achieved, this S protein is more likely to fall apart entirely owing to premature shedding of its S1 domain. Conversely, with G614, more energy is required to achieve a one RBD–up state, but dissociation of S1 from S2 also becomes less favorable because the remaining folded 630 loops continue to hold the trimer together. Thus, the D614G variants have more S proteins in the up orientation because the next, irreversible step toward inactivation is slower. Infection with D614G is more efficient because it prevents premature S1 shedding (see the figure).

These structural studies have real-life implications. All current vaccines are based on the original, unstable D614 form of the S protein (*13*). Fortunately, most vaccine developers, including Moderna and Pfizer-BioNTech, took a lesson from studies of SARS-CoV-1 and Middle East respiratory syndrome (MERS) coronavirus to slow S-protein shedding by introducing non-native prolines into S2 (*14*). Those who developed the Johnson and Johnson and Novavax vaccines had the prescience to also remove the furin site. By contrast, the developers of the University of Oxford–AstraZeneca vaccine opted for the wild-type S protein (containing D614), as is also the case for the inactivated virus vaccine produced by Sinovac. To be clear, other variables, especially antigen-delivery systems, likely account for efficacy differences among these vaccines. However, apples-to-apples studies in animals make clear that both engineered prolines and furin-site ablation contribute to vaccine effectiveness (*15*). It is almost certain that the next round of vaccines, those better reflecting the S-protein variants now in circulation, will include D614G. Vaccines that express unmodified S proteins with G614 may enjoy a relative jump in potency because this change compensates for the lack of engineered stabilizing mutations.

The work of Zhang *et al.* also reveals more about the natural history of the virus. The notable emergence of D614G suggests that the acquisition of a destabilizing furin site was a recent event. The virus could easily lose this site, as it does frequently in cell culture systems, implying that it in some way facilitates human transmission. This is not a conclusion that most students of human coronaviruses would have anticipated, given that SARS-CoV-1, which transmits with reasonable efficiency, lacks this site, whereas the more distantly related MERS coronavirus bears this site and transmits poorly. How the SARS-CoV-2 furin site promotes new human infections remains a key open question in the field. ■

Enhancing viral transmission

The Gly^{614} (G614) mutation in spike (S) increases ordering of the 630 loop compared with wild-type Asp^{614} (D614). This prevents the premature S1 shedding often seen with wild-type S proteins, ensuring that more S protein remains in a fusion-ready "one-up" state, with one receptor binding domain (RBD) exposed within the trimer, ready to bind angiotensin-converting enzyme 2 (ACE2) on host cells, increasing infection efficiency.



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