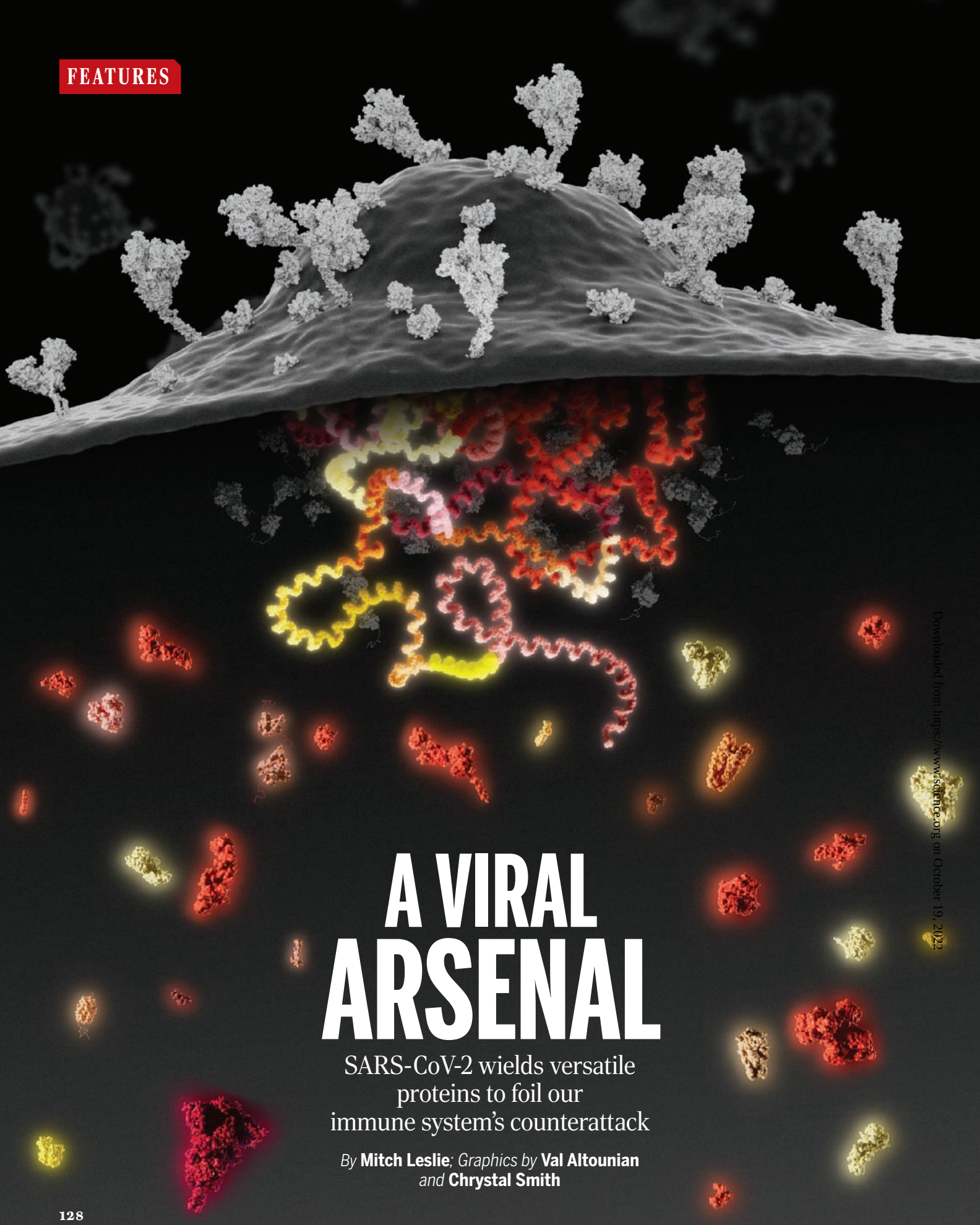


A VIRAL ARSENAL

SARS-CoV-2 wields versatile proteins to foil our immune system's counterattack

By **Mitch Leslie**; *Graphics by* **Val Altounian** *and* **Chrystal Smith**

Alpha, Beta, Delta, Omicron, BA.5—with each new SARS-CoV-2 variant or subvariant, the coronavirus seems to hone its ability to infect and spread between people. Although vaccines, drugs, and immunity from prior infections are allowing more and more people to dodge severe cases of COVID-19, the coronavirus has already killed more than 6 million people, according to the World Health Organization, and the true toll may exceed 18 million. Some virologists worry COVID-19 is here to stay, with SARS-CoV-2 potentially sickening people once or more a year as adenoviruses and other coronaviruses that cause the common cold do.

One key to the virus' success is its ability to neutralize the body's immune response, thanks to its arsenal of proteins. Over the past 3 years, investigators have begun to explore those viral countermeasures. They've shown that many of SARS-CoV-2's molecules manage to shield the virus—at least temporarily—from host immunity, allowing the invader to replicate and spread to more people.

A pitched battle between virus and host erupts when SARS-CoV-2 invades vulnerable body cells, transforming them into virus factories. The incursion, initiated when the spike proteins on the virus latch onto cellular targets, trips an alarm, provoking a sweeping counteroffensive by the multipronged immune system. Cells that are under attack begin to release potent immune proteins called interferons that boost resistance to the coronavirus. Once certain immune soldiers called CD8-positive T cells detect signs of SARS-CoV-2, they hunt down and destroy infected body cells, reducing the production of new viruses. Other immune warriors known as B cells begin to churn out antibodies that glom onto virus particles, locking them out of cells.

All too often, the virus manages to thwart these defenses. As it replicates ferociously in millions of people, its spike protein has picked up mutations that allow the virus to evade neutralizing antibodies evoked by vaccines or previous infection.

And like many viruses, SARS-CoV-2 is adept at blocking, dodging, and deceiving our immune protections in other ways. "Viruses are usually in an arms race with the host," says viral immunologist Adriana Forero of the Ohio State University College of Medicine. Their strategies elicit grudging admiration from virologist Stanley Perlman of the University of Iowa, who has been studying coronaviruses for more than 30 years. "Every time we discover something really cool in the immune system, we find a virus has already counteracted it," he says.

After SARS-CoV-2 infects a cell, it releases its RNA genome, which prompts the production of proteins that build fresh viruses and disrupt cellular defenses.

POTENT PROTEINS

Underpinning SARS-CoV-2's counterattack is the versatile suite of proteins that it coerces infected cells to manufacture from its RNA code. Researchers still disagree about how many proteins the virus' cellular victims make—estimates range from 26 to more than 30—but SARS-CoV-2 deploys more weapons than most other RNA viruses. The Ebola virus, for example, makes do with only seven proteins.

To probe how a particular SARS-CoV-2 protein upends our immune defenses, researchers typically engineer cells to produce unusually large amounts of the molecule. They then catalog the effects on cellular responses such as interferon output. Such studies suggest most proteins in the virus' arsenal play immune suppressing roles—even such unlikely actors as the membrane protein that helps new viral particles assemble and the editing enzyme that snips freshly made proteins down to size.

SCIENCE ONLINE

An enhanced version of this feature allows readers to examine how each SARS-CoV-2 protein may sabotage host defenses. Go to https://scim.ag/ViralArsenal.

Not all of these functions have been confirmed, however. Perlman cautions that overproducing just a single viral protein might not trigger the same cellular effects as a natural infection by the virus. Verifying the findings will require experiments with the virus itself, genetically tweaked to lack individual proteins, but researchers have performed few such studies, which require elaborate biosafety precautions. Immune-suppressing proteins may also rely on partnerships with one or more other viral molecules, virologist Susan Weiss of the University of Pennsylvania, Perlman, and colleagues recently revealed in a study of MERS-CoV, the relative of SARS-CoV-2 that causes Middle East respiratory syndrome.

It's already clear, however, that SARS-CoV-2 goes to great lengths to sabotage the body's interferon response, which is central to our defense against viruses. Interferons switch on hundreds of genes that can stymie every step in a virus' infection cycle. Some fortify cells' outer defenses and enable them to rebuff viruses that try to break in. Others boost the internal defenses of cells that become infected, curbing the production of viral molecules or preventing them from assembling into new viral particles. Still other interferon-stimulated genes stop newborn viruses from leaving an infected cell. Interferons also help recruit T and B cells to the body's battle with the virus.

Studies of COVID-19 patients have underscored the importance of interferons for fighting off SARS-CoV-2. For example, interferon responses are faulty in a sizable percentage of patients with severe disease. Up to 20% of the sickest people carry antibodies that latch onto and incapacitate their own interferons, researchers have found.

Many other pathogens, including the viruses that cause flu, Ebola, and hepatitis C, take aim at the interferon response. But SARS-CoV-2 stands out, Forero says. "What's unusual is how comprehensive the virus is." Its various proteins disrupt multiple steps, including a cell's detection of viral RNA, transmission of the alert signal to the nucleus, synthesis of interferons, and activation of interferon-stimulated genes. Moreover, multiple coronavirus proteins can block the same step.

Some SARS-CoV-2 proteins use trickery to circumvent the interferon response. Nonstructural protein 15 (Nsp15), for example, snips distinctive sequences out of newly made viral RNA molecules, helping disguise the RNA from cellular pathogen detectors that would otherwise trigger interferon production. Even some proteins whose main role is structural join the counteroffensive. For example, the nucleocapsid protein's day job is packing the pathogen's RNA into the interior of the viral particle. But a study this year revealed a cellular enzyme snips the nucleocapsid protein into fragments that can block interferon production by infected cells.

SARS-CoV-2 doesn't just interdict the interferon response, however. It may also stymie other immune defenses. Some studies suggest, for example, that viral proteins such as ORF3a, ORF7a, and the envelope protein hobble a process called autophagy, in which infected cells digest their own contents, breaking down viruses and individual viral proteins in the process. SARS-CoV-2 may also meddle with MHC-I, a protein that displays bits of the invader on the surface of infected cells and summons T cells. Viral proteins such as ORF6 and ORF8 may curb cells' production of MHC-I or block its transfer to the cell surface, preventing T cells from recognizing and killing infected cells.

MULTITASKING MOLECULES

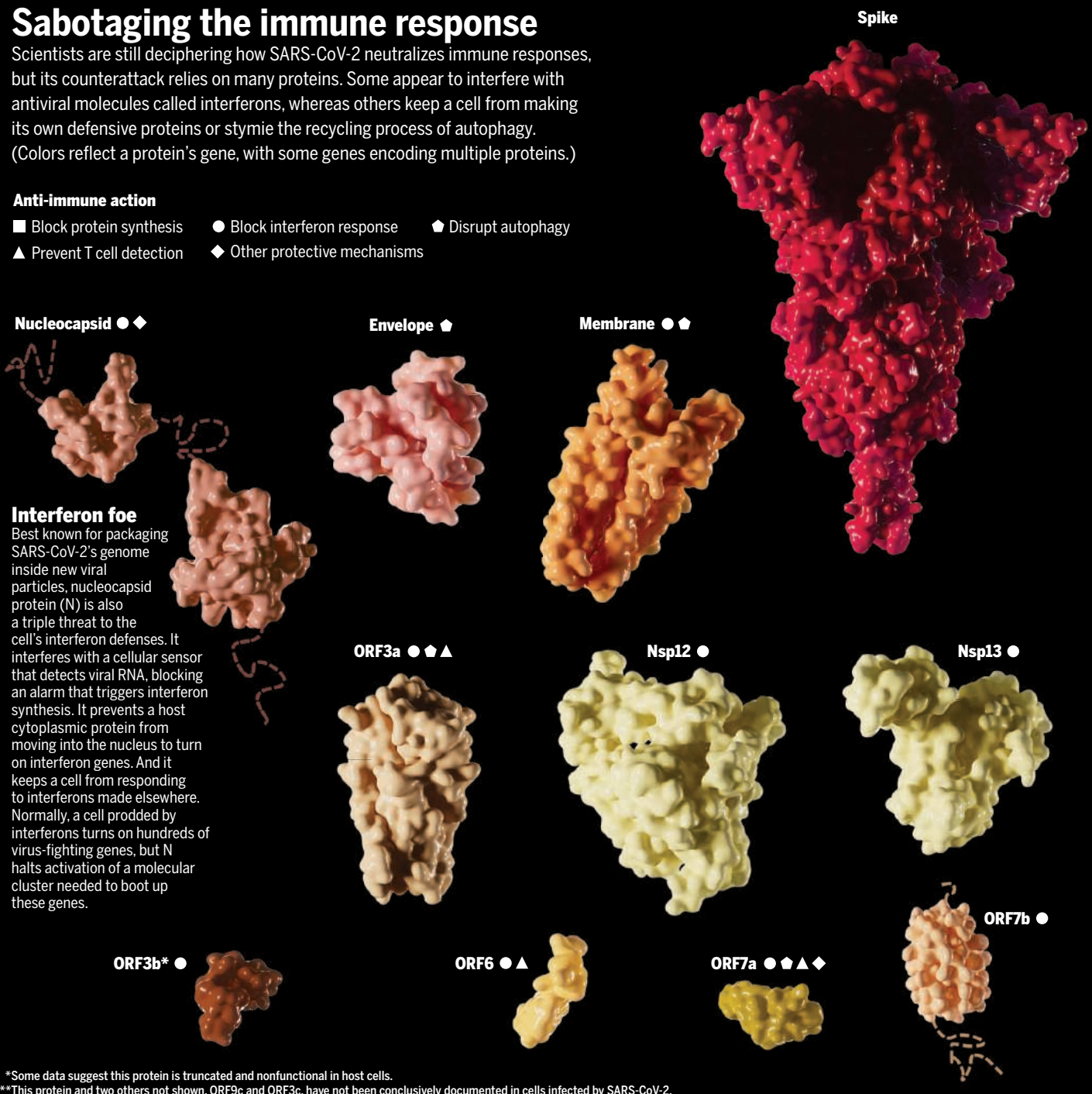
The virus gets the most from its small repertoire of proteins, many of which do double or triple duty. Nsp14 typifies these multitasking molecules. Like many of the virus' other proteins, Nsp14 performs several tasks that have nothing to do with immune eva-

Sabotaging the immune response

Scientists are still deciphering how SARS-CoV-2 neutralizes immune responses, but its counterattack relies on many proteins. Some appear to interfere with antiviral molecules called interferons, whereas others keep a cell from making its own defensive proteins or stymie the recycling process of autophagy. (Colors reflect a protein's gene, with some genes encoding multiple proteins.)

Anti-immune action

■ Block protein synthesis ● Block interferon response ⬟ Disrupt autophagy
▲ Prevent T cell detection ◆ Other protective mechanisms



Interferon foe

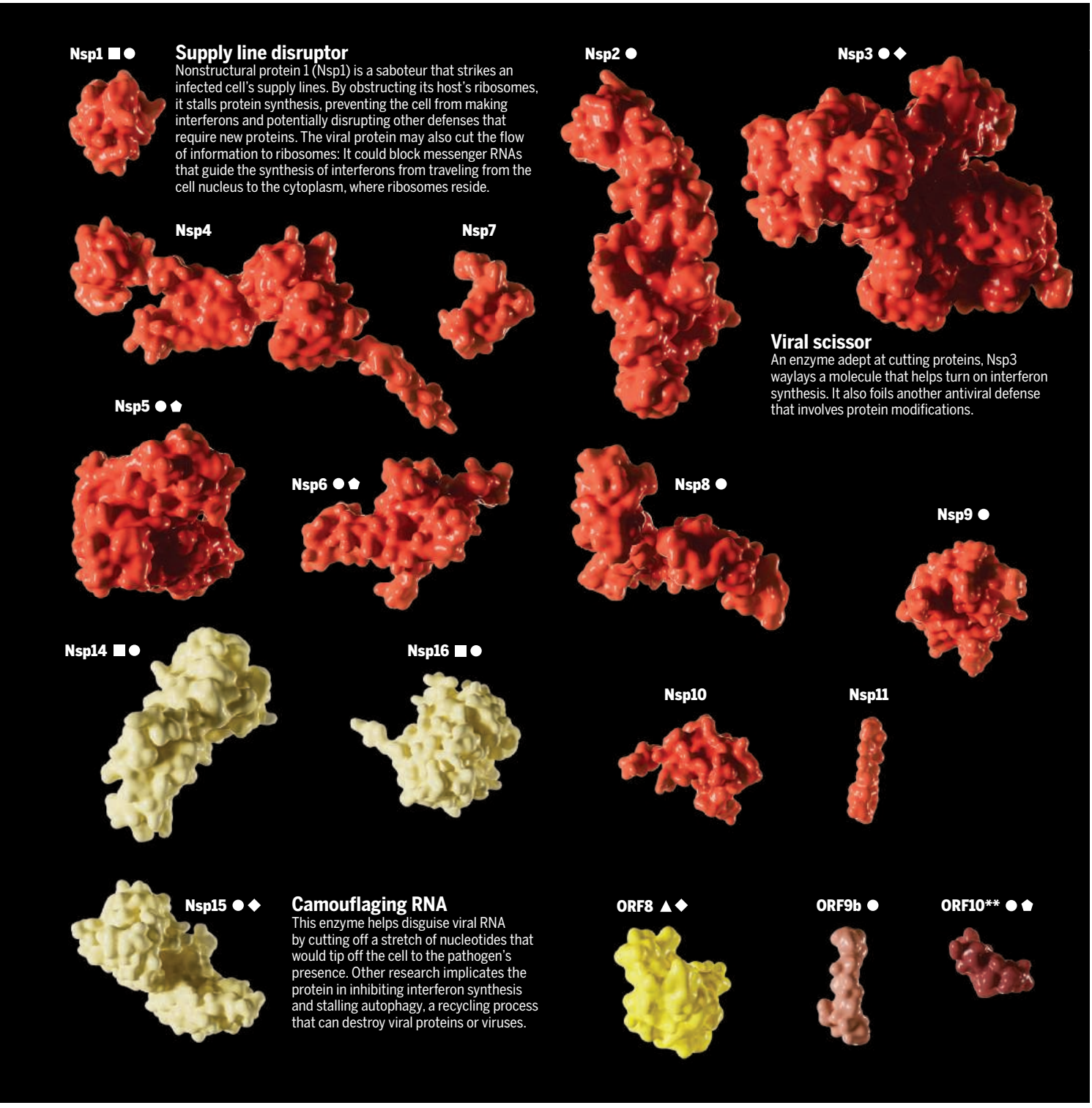
Best known for packaging SARS-CoV-2's genome inside new viral particles, nucleocapsid protein (N) is also a triple threat to the cell's interferon defenses. It interferes with a cellular sensor that detects viral RNA, blocking an alarm that triggers interferon synthesis. It prevents a host cytoplasmic protein from moving into the nucleus to turn on interferon genes. And it keeps a cell from responding to interferons made elsewhere. Normally, a cell prodded by interferons turns on hundreds of virus-fighting genes, but N halts activation of a molecular cluster needed to boot up these genes.

*Some data suggest this protein is truncated and nonfunctional in host cells.
**This protein and two others not shown, ORF9c and ORF3c, have not been conclusively documented in cells infected by SARS-CoV-2.

CREDITS: (GRAPHIC) V. ALTOUNIAN AND C. SMITH/*SCIENCE*; (PROTEINS) HTTPS://SCIM.AG/VIRALARSENAL

sion. It teams up with Nsp10 to help the virus reproduce by correcting errors in newly synthesized copies of the viral RNA genome. Nsp14 also combines forces with other proteins, including Nsp9 and Nsp12, to tag the RNA with a molecular cap that allows ribosomes, the cell's proteinmaking factories, to read it and churn out viral proteins.

Those jobs should keep Nsp14 busy. But a 2021 study by immunologist Jack Chun-Chieh Hsu of Yale University and colleagues revealed yet another possible function: The molecule somehow shuts down an infected cell's production of its own proteins. Hsu says this blockade may benefit SARS-CoV-2 by forcing a cell to divert its resources into making viral proteins. It may also prevent the cell from turning on interferon-stimulated genes. "It's an important strategy to counter the production of these antiviral proteins," Hsu says. Another of SARS-CoV-2's weapons, Nsp1, also appears to induce a similar immunity-blocking effect by jamming ribo-



somes, keeping them from producing the host cell's own proteins. For reasons scientists don't understand, though, the cell is still able to make viral proteins.

Researchers may be able to turn their knowledge of SAR-CoV-2's anti-immune tactics against the virus. Several groups have screened existing compounds and drugs for activity against Nsp15. So far, however, no one has created drugs specifically designed to thwart SARS-CoV-2's anti-immune effects. (Pfizer's drug combo of nirmatrelvir and ritonavir, better known as Paxlovid, targets a viral enzyme, Nsp5, which has immune-thwarting properties, but the company's goal was instead to block the protein's central role in SARS-CoV-2's replication.)

Scientists don't yet have the full picture of SARS-CoV-2's ploys for eluding the body's defenses, but as the pandemic drags on, they will have plenty of opportunities—and plenty of urgency—to learn more. "We have built a nice foundation," Forero says, "but there is a lot of room to go further." ■

A viral arsenal

Mitch Leslie

Science, 378 (6616), • DOI: 10.1126/science.adf2350