New insights into the structure of the influenza virus hold promise for better vaccines and antivirals.
The scientific textbook depiction of the flu virus is getting a facelift. A Pitt team has discovered that a model of the influenza genome architecture, untouched since the 1970s, isn’t so perfect after all.

The discovery, reported in Nucleic Acids Research in July, reveals loopholes in the way the virus packages its genetic material. When one strain of flu comesling with another strain inside a cell, these loopholes allow the viruses to swap genetic material and give rise to new strains of flu. Knowing about these loopholes and how they interact with one another could give scientists the opportunity to better predict pandemics and find new ways to disrupt the flu virus.

“We know surprisingly little about flu pandemics.”

Although influenza has plagued mankind for hundreds of years and poses a substantial public health threat every winter, we know surprisingly little about flu pandemics,” says senior author Seema Lakdawala, a PhD assistant professor of microbiology and molecular genetics. “Our discovery may give insight into how the flu virus continually evolves, opening the door to better vaccines and antivirals.”

Influenza is a type of virus that uses single-stranded ribonucleic acid (RNA) to replicate, instead of double-stranded DNA. Influenza viruses are made up of eight RNA segments bound by a protective nucleoprotein. All eight RNA segments must come together inside a virus particle to make it fully infectious.

The classic model of the flu virus has these proteins coating the RNA like beads evenly spaced along a string. However, limitations of techniques used in the 1970s, when the model was developed, meant that unique features—like exposed RNA loops—were not apparent. Consequently, the universal depiction of influenza in textbooks is of a uniform random binding of proteins along the entire length of each RNA segment.

Lakdawala, who researches how viruses emerge and spread, teamed up with lead author Nara Lee, a PhD assistant professor of microbiology and molecular genetics, who specializes in RNA interactions. The two were curious whether there might be any areas along the influenza RNA strand that are more “open” and, therefore, more able to associate with other RNA segments. They used a process called “high-throughput sequencing of RNA by crosslinking immunoprecipitation” on two strains of influenza A, including the 2009 H1N1 pandemic (swine flu) strain, to get a better understanding of where the proteins bind to the RNA and to see if there were any areas of “naked” RNA.

“Honestly, we didn’t expect to find any, since we had all learned the ‘beads on a string’ depiction of viral RNA,” says Lakdawala. “But, amazingly, there are several stretches where the RNA was not bound by the nucleoprotein. This discovery opens up a whole new area of research.”

Contrary to the classic model, Lakdawala and Lee found there are areas of RNA rich with protein coating and others that are exposed and presumably ripe for binding to other viral RNA during reassortment or to the swapping of genomic material between flu viruses. Evolutionary biology expert Vaughn Cooper, a PhD associate professor of microbiology and molecular genetics, guided the team to explore how these loops shape virus evolution in nature and during normal flu seasons.

The team is pursuing several potential research opportunities, including predicting the ways different influenza viruses could share genetic material to make new viruses. Knowing this could point scientists to the reassortments most likely to spark a flu pandemic and give public health agencies a leg up on creating targeted vaccines. There also could be ways to exploit the exposed RNA to make the virus less transmissible and deadly.

“It’s really exciting to suddenly have all these research possibilities open up based on this one discovery,” says Lakdawala. “The reason no one uncovered this [before] is because we all took for granted that 50-year-old research on the genome architecture, which looked really nice and had an easy explanation, was the full story. It shows that if we don’t constantly resample and question scientific dogma, we could miss a big opportunity.”
When a child is born with a condition known as hypoplastic left heart syndrome (HLHS), the family faces daunting challenges.

First, the baby must undergo a three-part surgery to correct life-threatening heart deformities. Then, if the heart still doesn’t pump properly, the child goes on a heart transplant waiting list, and the family has to hope a donor will be found in time.

Recently, researchers at the University of Pittsburgh led by Cecilia Lo discovered a genetic cause of HLHS in mice, raising the possibility that there might one day be drugs that could at least partially correct the heart defect.

Lo, a PhD who holds the F. Sargent Cheever Chair of Developmental Biology, and her colleagues screened 100,000 mice with chemically mutated genes and looked for animals showing the classic signs of HLHS—a severely underdeveloped left ventricle, which normally pumps blood through the body, and a narrowed aorta, the largest artery in the body.

Of those 100,000 mice, eight fit the bill. The team then compared the genomes of these eight strains to those of healthy controls and found more than 300 mutations in the mice with heart defects. Further analysis showed HLHS was likely caused by combinations of many interacting genes.

The team soon zeroed in on one combination in particular, Sap130 and Pcdha9—in one of the mouse strains, mutations in both genes were required for all the hallmarks of HLHS to develop. Variants of Sap130 appeared to cause the small left ventricle and a weakness in heart metabolism, Lo says, while variants of Pcdha9 affected the aorta.

Lo’s team collaborated with Stephen Murray and Kevin Peterson at the Jackson Laboratory in Maine, experts in the new genetic manipulation technique known as CRISPR-Cas9. Using this technique of altering genes in healthy mice, they were able to show these two mutations, once again, causing HLHS.

Working with colleagues at the University of Cincinnati and the University of California at San Diego, the team also examined a small sample of 68 patients with HLHS; one had Sap130 and Pcdha9 mutations, which are both rare.

The work, published in the May 22 issue of Nature Genetics, details what may be the first known genetic cause of HLHS, which affects about 2 to 3 of every 10,000 live births, or about 1,200 babies per year in the United States.

Eventually, Lo says, understanding the genetics of the syndrome might allow doctors to at least partially counteract the maldevelopment of an infant’s heart with drug treatments. Most babies with HLHS are now identified in the womb, and it’s possible that future therapies might reverse or reduce the severity of these malformations before the child is born.

“If we understand what causes this small left ventricle—and you can somehow treat that in utero—you can make the left ventricle grow . . . big enough that you can support biventricular physiology,” says Lo. Then, doctors could do surgery after the babies are born and “still have two functional chambers [in the heart]. This would improve the patient’s long-term prognosis.”

The research also might allow doctors to one day determine which HLHS patients have abnormally weak heart muscles and therefore won’t benefit from surgical repair; this would allow such children to get on the transplant list more quickly. “A number of studies have shown that the earlier you get a transplant, the better your outcome,” she says.

It is likely that other combinations of mutations cause HLHS in the mouse strains the team worked with, Lo notes; so she is now seeking funding to search for other culprits responsible for this difficult congenital condition.
Researchers had already figured out in great molecular detail how immune cells recognize and attack foreign tissue—a mechanism that leads the body to reject about half of all organ transplants. But one thing wasn’t clear: What immune system signal kick-starts the process?

This summer, a Science Immunology paper by Fadi Lakkis provided an answer to this longstanding stumper. “What we’ve discovered is a very fundamental mechanism,” says Lakkis, the Frank and Athena Sarris Professor of Transplantation Biology and of surgery, immunology, and medicine, as well as scientific director of the Thomas E. Starzl Transplantation Institute at the University of Pittsburgh.

Mammalian immunity has two lines of defense: innate immunity, a primitive system consisting of tissue barriers and cells that are permanently mobilized to fight whatever toxins and pathogens they encounter, and adaptive immunity, the more recently evolved system that deploys a dragnet of T lymphocytes and other cells that search out and destroy specific interlopers when the innate immune response can’t do the job.

It’s the adaptive immune system that engineers transplant rejection. Unless a patient receives heavy doses of immune-suppressing drugs, the adaptive immune system identifies replaced organs as foreign tissue. But the adaptive immune system’s marching orders come from the innate immune system, which hasn’t generally been thought to be able to distinguish self from nonself tissue. Historically, researchers have assumed that generalized inflammation was sparked when a failing organ was removed and a new one plopped into its place, bringing T cells to attention.

But Lakkis was bothered by one truth of biology that holds up across species: Only vertebrates have adaptive immune systems, and yet invertebrates—which evolved earlier—can also distinguish their own tissues from foreign ones. “This was a very strong hint,” he says, “that perhaps the innate immune system had some nonself recognition chops that had gone underappreciated.

Lakkis teamed up with Matthew Nicotra, the PhD assistant professor of surgery and immunology at Pitt whose work had illuminated this ability to distinguish self from foreign matter, called allorecognition, in invertebrates. The duo searched for similar signals in the innate immune systems of mice. Using a genetic technique called positional cloning, they homed in on a molecule called SIRP-alpha and a receptor it binds to called CD47. SIRP-alpha is an ideal nonself marker because different people have slightly different versions of it. The researchers conducted a series of transplant experiments in mice and found that when a slightly divergent SIRP-alpha in donor tissue engaged with the host’s CD47, the innate immune system revved up.

The team’s first priority is to make sure the finding extends to humans. If so, then the signaling system might explain why, even years after a successful transplant, an organ is still at risk of rejection. “To us, it indicates that something is constantly waking up the innate system,” Lakkis says, “and then the innate system turns around and wakes up the adaptive immune system.”

Interfering with this signaling could help prevent rejection, Lakkis says, though he speculates that the system comprises much more than what they’ve found so far. “My guess is that we have just discovered the tip of the iceberg,” he says. “There may be many other molecules that are activating the immune system in a similar fashion.”