By JULIE KIEFER

Researchers' Portraits
by JAY A. BOROWCZYK

Looking for HIV in all the right places:

Wesley I. Sundquist, Ph.D.

The Serendipitous Science of Sundquist and Hill

While hiking in the Wasatch Mountains aglow in fall splendor, Wesley I. Sundquist, Ph.D., and Christopher P. Hill, Ph.D., chatted about science, politics, and the hiring process. Sundquist, then the newest member of the medical school's Department of Biochemistry, had been given the task of convincing Hill to join the faculty. The outing not only kindled a friendship, but also a very successful, long-term scientific collaboration.

Fifteen years later, Sundquist and Hill are full professors and world-renowned human immunodeficiency virus (HIV) researchers. They'll soon be working together at a new Structural Biology Center for HIV/Host Interactions in Trafficking and Assembly to be established at the University of Utah with a $19.2 million, five-year grant from the National Institutes of Health (see pg. 28).

"Wes and Chris clearly lead the field of HIV structure determination. This award is the latest manifestation of how well they're regarded by their colleagues," said Dana Carroll, Ph.D., professor and chair of the Department of Biochemistry. "The more we understand about how HIV infects cells and causes disease, the more opportunities we have to intervene either prophylactically or in treatment."

Much of the work by the U researchers impacts the problem of drug resistance among sufferers of autoimmune deficiency syndrome (AIDS), the disease caused by HIV. Just as infectious bacteria have evolved to tolerate commonly prescribed antibiotics, HIV is becoming resistant to existing drugs that keep it in check.

About 15 percent of new HIV infections are drug-resistant, a number that will only grow as the rogue virus spreads among the population, according to Sundquist, who holds the H.A. and Edna Benning Presidential Endowed Chair in Biochemistry. "If we don't come up with new drugs," he warns, "there is going to be a severe problem."

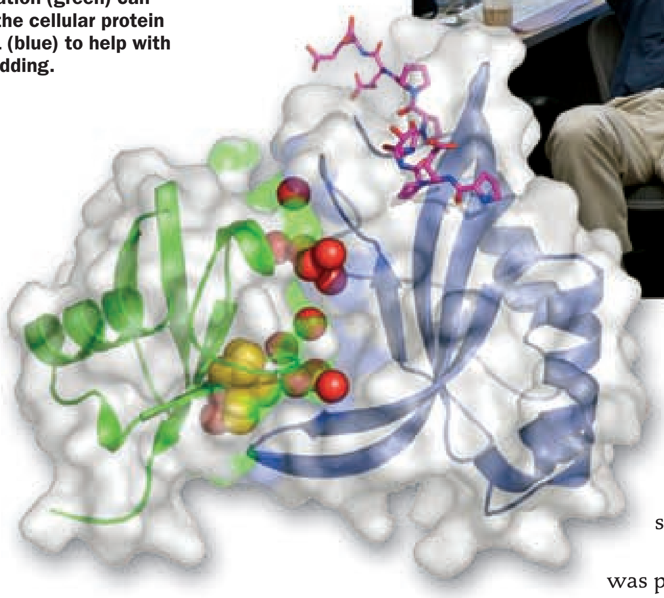
The investigators are approaching the problem from two sides. Sundquist uses biochemistry to identify proteins that are important for the HIV life cycle and determine what their jobs are. Hill, using a technique called X-ray crystallography to build 3-D models, or structures, of the proteins, figures out how these proteins do their jobs. "Knowing how a motor works involves seeing all the pieces," Sundquist noted. Contributions from both researchers have proven invaluable for understanding how HIV works.

In collaboration with Myriad Genetics in Salt Lake City, Sundquist exposed one of HIV's attack strategies. Our cells normally use specialized machinery—the multivesicular body (MVB) pathway—to bring unwanted proteins into a specialized cellular compartment prior to destruction. The MVB machinery forces the membrane surrounding the compartment to bulge inward and pinch off, creating a sphere or "vesicle" that envelops the waste. Sundquist determined that HIV coerces the cell's MVB complex into encasing viral proteins in vesicles. In this case, membranes are forced to bulge out of the cell. After pinching off, the virus is released and free to infect more cells.

Sundquist determined that HIV is dependent on certain MVB proteins, including one called Vps4, to spread. When he introduced mutations into these proteins, resulting HIV-containing vesicles remained stuck to the cell surface like pimples. Because they failed to "bud," the virus could not infect additional cells. These landmark findings earned Sundquist the 2003 American Society for Biochemistry and Molecular Biology-Amgen

Christopher P. Hill, Ph.D.

Below, a structural model showing how the HIV Gag protein (red) and its ubiquitin modification (green) can recruit the cellular protein TSG101 (blue) to help with virus budding.



Award for significant achievements in the understanding of disease.

Building upon Sundquist's foundation, Hill investigated how Vps4 works. After the vesicle is made, Vps4 detaches the rest of the MVB complex from the membrane. Hill determined that part of Vps4 is shaped like a donut and has an "arm" that extends into the donut's hole.

"Sometimes you see a structure and it immediately tells you how, at some level, the system works," said Hill. The structure suggested that Vps4 uses its arm to pull MVB machinery through its hole, breaking its hold on the membrane.

Surprisingly, studying HIV was not originally in Sundquist's plans. As an assistant professor, he intended to continue his postdoctoral work studying nucleic acids. When that research unexpectedly came to a dead end, he decided to shift his focus. After much reading, he realized that HIV's "structural" proteins, those that house the virus' genetic material, were vastly understudied.

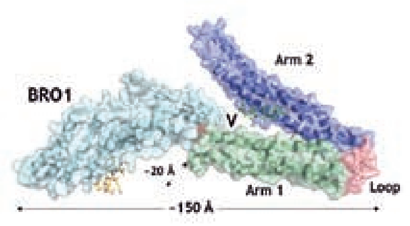
"My decision to research HIV wasn't some sort of 'dying to cure AIDS' epiphany," admitted Sundquist. "Science is more serendipitous than people realize."

Like Sundquist, Hill never intended to work on HIV. However, he was primed to do so. Hill started his graduate studies at the University of York, England, in the mid-1980s, just after the discovery of HIV. A group there was part of a nationwide initiative to defeat the virus. Although Hill wasn't directly involved in the work, he was eager to learn about it and actively participated in its scientific discussions.

"I had given HIV quite a bit of thought beforehand, but needed a collaborator to go with it," explained Hill. "So when Wes asked if I wanted to help him with the HIV project, I was in there like a rat up a drain."

Their stories illustrate a guiding principle shared by Sundquist and Hill. "It's more about recognizing opportunities and making the correct decisions as they arise than it is about long-term planning," said Sundquist. The researchers' "take-it-as-it-comes" outlook continually influences the direction of their research.

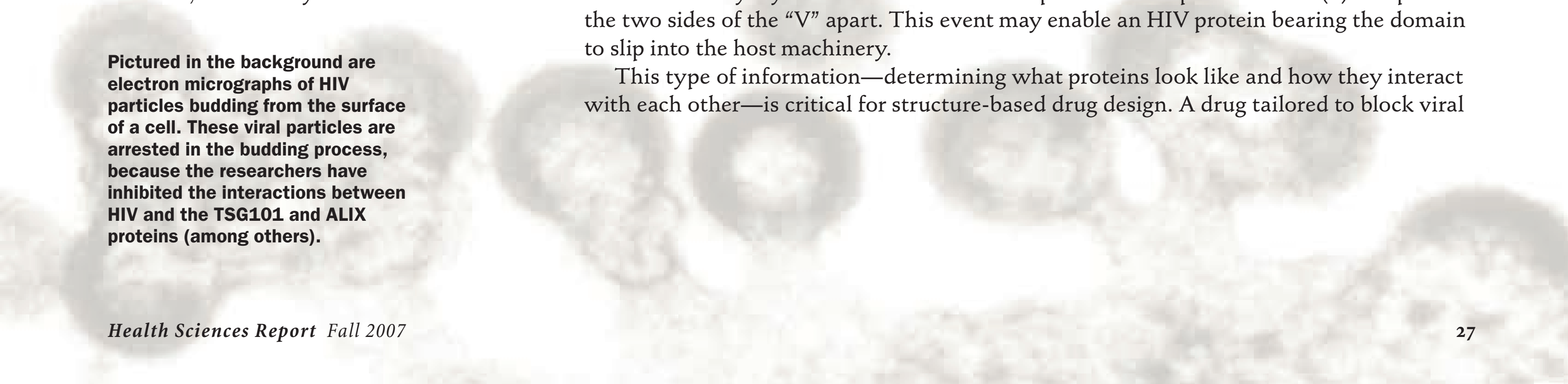
Sundquist's ongoing work on the MVB machinery gave him insights into HIV's invasion (replication) strategy. He felt he couldn't pass up the opportunity to investigate further. HIV disguises itself as a cellular protein by evolving two "late domains," called PTAP and YPX(3)LXL. These are like membership cards that provide access to the MVB pathway through one of two entrances. Sundquist helped discover that proteins wielding PTAP or YPX(3)LXL can exit the cell via the MVB proteins, TSG101, and ALIX, respectively.



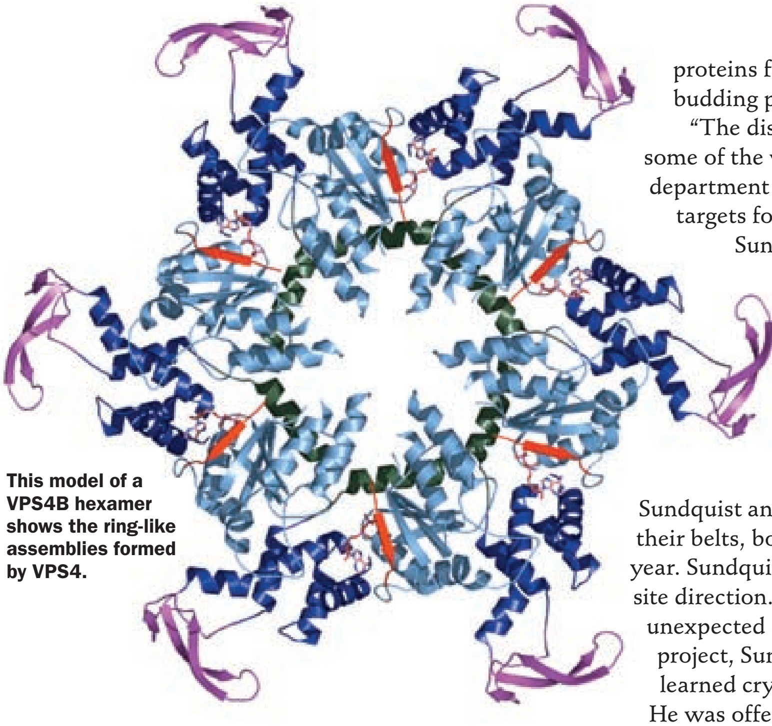
Structure of the cellular ALIX protein. This is the other known cellular protein that binds directly to Gag and connects it to the cellular budding machinery.

To gain insight into how ALIX works, Hill solved its structure. He found that part of ALIX is shaped like a "V" and that YPX(3)LXL fits lock-and-key style into its crevice. The shape of ALIX implies that YPX(3)LXL pushes the two sides of the "V" apart. This event may enable an HIV protein bearing the domain to slip into the host machinery.

This type of information—determining what proteins look like and how they interact with each other—is critical for structure-based drug design. A drug tailored to block viral



Pictured in the background are electron micrographs of HIV particles budding from the surface of a cell. These viral particles are arrested in the budding process, because the researchers have inhibited the interactions between HIV and the TSG101 and ALIX proteins (among others).



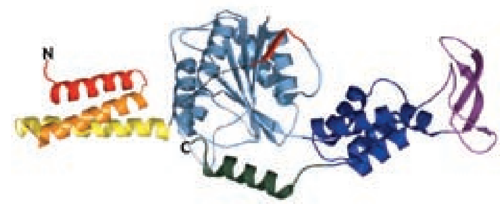
This model of a VPS4B hexamer shows the ring-like assemblies formed by VPS4.

proteins from accessing the cell's vesicle machinery can cripple the budding process and prevent the spread of HIV.

"The discoveries that Chris and Wes have made about the structure of some of the viral components can be turned into a new class of drugs," said department chair Carroll. "The structural components provide additional targets for drug development or intervention of some kind."

Sundquist is cautious when making predictions, however. "I believe in the importance of what Chris, I, and our colleagues are doing, and that our work can lead to useful new therapies. But I think it's very important that we avoid 'over-hyping' our work—particularly on a virus that kills about 3 million people every year. Any solutions coming from us and others will likely take time and be implemented in a piecemeal fashion."

Considering their similar backgrounds, it is no wonder Sundquist and Hill work so well together. With chemistry Ph.D.s under their belts, both moved overseas to work in crystallography labs in the same year. Sundquist moved from the U.S. to the U.K.; Hill traveled in the opposite direction. Due to an unexpected change in his project, Sundquist never learned crystallography. He was offered a position at Cambridge, but opted to return home to the U.S. Hill's search for a scientifically exciting environment allowed him to consider more options. They were both offered jobs at the University of Utah. It was the right fit.



This is the structure of the VPS4 protein, which uses the energy of ATP to help the virus bud.

"There was a genuine enthusiasm for science," said Hill. "I thought it would be a good collaborative environment and a great place to grow and to build something."

"The biochemistry department was a very special place," agreed Sundquist. "There was very high quality science and great collegiality among the faculty."

As the newest faculty in the department, the two quickly became each other's support system. Hill lived with Sundquist while searching for an apartment and worked in Sundquist's lab while his was being built. During those first years, "we were both teaching, writing grants, recruiting students, and directing research projects for the first time. We served as mentors for each other," said Hill. Their shared experiences cemented their camaraderie.

Familiar with the power of crystallography, it was natural for Sundquist to ask Hill to collaborate on his new HIV project. "One decision I made early on was that I wasn't going to work on HIV except in collaboration with Wes," Hill recalled. "I didn't want to get into a competitive situation. That would've been untenable. I think that was quite an important decision to make for the long-term viability of the collaboration and our friendship."

Their families have also become friends, and they get together for barbecues, hikes, and camping. The scientists take advantage of these gatherings to hash out ideas in a relaxed atmosphere.

"Initially, we don't always agree about interpretation of experimental results," said Hill. "We work together to sharpen ideas against each other. 'How do you know this? What makes you say that?' We act as a foil for each other."

"Our collaboration has worked very well. My other collaborations aren't at the same level—they haven't been going for 15 years," said Sundquist. "I'm very fortunate to have Chris as a friend and colleague." ◩

For more information:

Drug resistance in HIV
http://www.who.int/drugresistance/en/
http://www.thebody.com/content/treat/art2836.html

Chris Hill, Ph.D.
https://wasatch.biochem.utah.edu/chris/

Wes Sundquist, Ph.D.
https://wasatch.biochem.utah.edu/wes/

Sundquist Heads New HIV Structural Biology Center

"I worked on this day and night for two months," said Wesley I. Sundquist, Ph.D., letting a copy of a 300-page grant application fall on his desk. Then he smiled.

His hard work paid off last spring—big time.

Sundquist was awarded $19.2 million over five years from the National Institute of General Medicine, National Institutes of Health, to develop and direct a new Structural Biology Center for HIV/Host Interactions in Trafficking and Assembly. Studies will be conducted primarily at the University of Utah and The Scripps Research Institute in La Jolla, Calif.

One of the center's objectives is to study a new development in HIV research. Human cells have "intrinsic immunity," enabling them to fight the virus in its attempt to overtake the cell's machinery. Under selective pressure, the rapidly evolving virus has come up with evasive maneuvers to dodge the cell's every jab. Researchers will investigate this complex interplay between virus and host, with a potential outcome of identifying additional drug targets.

"This is one of the most exciting times in HIV research," said Sundquist. "It looks like inactivating any of the HIV proteins that overcome intrinsic immune pathways would have a profound effect on viral replication."