Brett Lindenbach: Deconstructing and reconstructing hepatitis C

Lindenbach built a new hepatitis C virus to find out how the wild one works. He plans to use this tool to understand the cell biology of virus–host interactions.

Hepatitis C virus (HCV), discovered almost 20 years ago, was resistant to being researched. Despite multiple attempts, researchers simply could not get this human pathogen to grow in culture. Lindenbach, as a young and enthusiastic graduate student had grand ideas about studying HCV, but fearing a dead-end project for a PhD student, his supervisor advised Lindenbach to work on the more willing yellow fever virus. The advice was wise; Lindenbach passed his PhD, after making yellow fever yield the secret of how a secreted protein could control viral replication inside the cell (1, 2).

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Lured by the success, Lindenbach started a postdoc studying other research-friendly viruses. But his satisfaction was shallow, and the puzzle of how to grow HCV was continually on his mind.

In 2005, Lindenbach cracked the puzzle. He built a new, artificial HCV (3) that could not only replicate in culture, but could also infect animals (4), proving that it was just like the real thing, only now researchable.

HIGH SCHOOL VIROLOGIST

When did you get interested in science?

I was interested in science at an early age, but both my parents were artists, so I didn't have a lot of exposure to science. My dad encouraged my interest, because he thought it was difficult to make a living as an artist—I don't think he realized that science is just as hard! But also, I think it was a way for him to live the scientific life vicariously—he had technical leanings. I actually became interested in viruses in middle school. I thought they were really cool. I remember reading a book that described the first genome ever sequenced. It was a small bacteriophage and its entire genome was written on half a page in the book. I thought to myself, "I can't believe that this is everything that little virus needs." It blew my mind.

Where did you go to college?

I went to the University of Illinois and majored in general biology. I was not sure what to do after college, but then I had a really great experience working as a tech in the laboratory of Ralph Wolfe. He studied archaebacteria, in particular methanogens, and I became fascinated. Not with methanogenesis per se, but with working in the laboratory. I decided I would go to graduate school and enrolled at Washington University in St. Louis. I studied for a PhD with Charlie Rice.

What was the focus of your PhD?

I wanted to study the interface between viruses and the immune system. Hepatitis C virus had just been discovered, and one of its major characteristics is that it causes chronic, persistent infections that are not cleared by the immune system. I thought that was a perfect topic.

But I didn't realize how hard HCV would be to study. I had to switch topics, and started working on yellow fever virus. This was the first human virus to be discovered, and there's a vaccine for it, so I was able to work with the live virus.

HCV GIVES IT UP

You now work on HCV. So when did you switch back?

After my PhD, I went off to do a postdoc in Madison, Wisconsin, with Paul Ahlquist, who was studying the model positive strand RNA viruses: Flock House virus and brome mosaic virus. They're really fantastic in that you can do all kinds of things with them that you can't do with other viruses, but I missed studying medically important viruses. Right around this time, Ralph Bartenschlager's group, who were working on HCV, made a breakthrough by developing replicons—a system for studying intracellular aspects of HCV replication.

Brett Lindenbach

Charlie had moved to Rockefeller [University] while I was in Madison, and he started working on these HCV replicons. He and I talked and decided that I should come back to his laboratory and work on developing the cell culture system further—the replicon system allowed us to study intracellular viral replication, but not the entire viral life cycle.

How do replicons differ from the normal HCV?

Replicons are positive strand RNAs, like the full virus, but they only encode a subset of the genes—the ones we think are important in RNA replication. They lack the structural genes that go on to make virus particles.

So what did you do with these replicons?

HCV has an extraordinary genetic diversity because of its error-prone replication, and most replicons can replicate in culture because they accumulate adaptive mutations. When I came back to Charlie's laboratory, we wanted to study full-length viruses in culture, but when we put the existing replicons into the full-length genome, we didn't get viral production. We worked on the hypothesis that the adaptive mutations in the replicons were inhibiting virus production.

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Around this time, another laboratory described a replicon called JFH that didn't need adaptive mutations to grow. We thought, "Aha, maybe if we make a full-length genome out of this replicon, it will make an infectious virus." So we contacted the laboratory, they sent us the replicon, we reconstructed a full-length genome, and lo and behold, it produced virus particles. We went on to show that the reconstructed virus was infectious in animals, so we knew it was bona fide.

Hang on, how does this reconstructed virus differ from the wild type?

That's a really good question. The JFH replicon was derived from a virus that came from a Japanese patient with fulminant hepatitis (that's where the name came from). But according to the researchers who derived the replicon, the original virus wouldn't grow in culture. The JFH replicon seems to be an unusual isolate that replicates without adaptive mutations.

Given HCV's genetic diversity, I'm sure there are additional virus strains out there that will also efficiently replicate in culture, we just haven't found them yet. So for the moment, reconstructed JFH strains are our best system for studying the complete life cycle of the virus.

So what have you learned about the virus's life cycle, using this construct?

When we first grew the virus, we looked at its buoyant density, or how well it floats, to learn about the basic physical properties of the virus. We found that HCV has two forms—a heavier form that's not very infectious, and a light infectious form. This form has an unusually low buoyant density, which is usually a sign that the virus is interacting with lipids.

Now we're interested in understanding, what exactly is in these particles? How does it contribute to the infectivity of the virus? And how are the different virus particles made? We think that the more buoyant form of virus might be coating itself with serum lipoproteins, to help mask it from the host immune response. But that's still an emerging concept.

A SHORT FUTURE FOR HCV?

What are you working on these days?

Actually, the reason I came to Yale was because of its strength in microbiology and cell biology. I'm in a section called Microbial Pathogenesis—basically a cross between a microbiology and a cell biology department. It's a small group of people, all devoted to studying the host–pathogen interaction at the cellular level, which has always been a strong interest of mine. So we've been doing some classical cell biological experiments to understand how virus particles are made.

We're also getting into real-time imaging of virus particles. And because we can manipulate the viral genome however we want, we can introduce tags that allow us to follow the viral proteins around.

By studying the virus–host interface, you end up learning more about each.

Oh, absolutely. I like to joke with some of my colleagues that cell biology is just a discipline of virology, because so many seminal findings had their origins in virology. May I remind you that the first article ever published in the *Journal of Cell Biology* was on imaging viruses?

In fact, some of the cellular pathways with which HCV interfaces are still poorly understood. For example, one of the small organelles involved in HCV assembly is lipid droplets, which were thought to be inert balls of fat. It turns out that they're actually dynamic structures that have all kinds of interesting properties. So, I'm sure we're going to learn more about lipid droplet biology, and other cell systems, as well as the virus itself.

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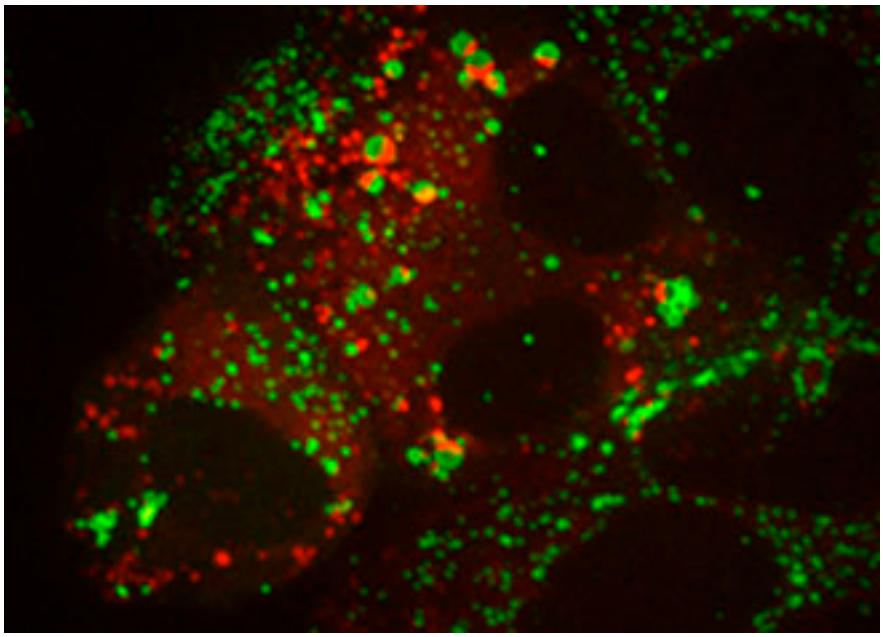
Where do you see yourself in ten years?

In ten years I should hope that we aren't working on HCV anymore, because it'll no longer be a problem! JCB

1. Lindenbach, B.D., and C.M. Rice. 1997. *J. Virol.* 71:9608–9617.
2. Lindenbach, B.D., and C.M. Rice. 1999. *J. Virol.* 73:4611–4621.
3. Lindenbach, B.D., et al. 2005. *Science.* 309:623–626.
4. Lindenbach, B.D., et al. 2006. *Proc. Natl. Acad. Sci.* 103:3805–3809.



Lipid droplets (green) are sites of HCV (red) assembly. Lindenbach expects to learn more about both viruses and their host cells thanks to live cell imaging studies.