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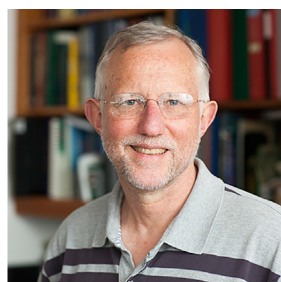
Bringing the Hepatitis C Virus to Life

Charles Rice and Ralf Bartenschlager, together with Michael Sofia, are the recipients of the 2016 Lasker~DeBakey Clinical Award for the their discoveries and development of a system to study the replication of hepatitis C virus, which causes a chronic and lethal disease, and for use of this system to invent drugs that cure the illness. Charlie and Ralf joined *Cell* editor João Monteiro in a conversation about their achievements and challenges and the future of HCV research.



Charles Rice
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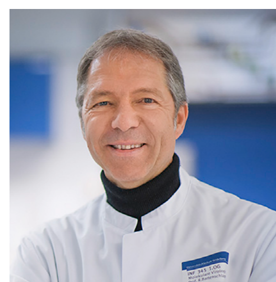

Ralf Bartenschlager
Heidelberg University

João Monteiro: I read your remarks for the Koch Award, which you also both shared last year, and I remember that you mentioned that, just because we have a drug to treat people with HCV now, that doesn't mean that this is the end of the road. Hepatitis C and its chronic complications are still a major public health issue. What are some of the remaining challenges for the field? Where should our attention be focused now?

Ralf Bartenschlager: I would say that, when it comes to challenges from a public health perspective, there are several challenges. The very obvious one is the pricing of HCV treatment. There is also the accessibility problem. When we are out in public talking about controlling HCV, this discussion is very often focused on those countries that can afford treatment. There are many countries that cannot, and if that doesn't change, they will not benefit as much from all of the progress that has been made. The second challenge is whether this would ever lead to an eradication of hepatitis C. Personally, I think that it is probably not realistic to expect that we will be able to eliminate HCV from the planet just by means of treatment—meaning that it is still worth the effort to develop a vaccine preventing transmission of the virus or at least chronic infections.

Also, a considerable number of patients do not benefit from current treatments, either because their viruses are not eliminated or because they still have a risk of developing liver cancer, a common complication of chronic HCV infection. There are still open questions with respect to the development of end-stage liver disease, especially hepatocellular carcinoma—whether or not, and after which time point of disease development, treatment may fail to prevent tumor development even though the virus has been cleared.

Charles Rice: I think Ralf has captured many of the challenges that we face, and it's not just issues of pricing and access, but also the infrastructure that it takes to efficiently implement advances like these new regimens that can essentially eliminate the virus, at least in the vast majority of treated patients. You would think that, once medicines are developed, there should be a fast track to implementing them on a scale that reaches those in need. I think that many of the hurdles that exist for HCV are public health challenges that we face with many diseases.

JM: From a basic biology perspective, I know that we've made a lot of progress, but do you see any particular outstanding questions that still need to be resolved about either the biology of the virus or how the virus leads to chronic disease?

CR: I think that there are a lot of them! Certainly, as much as we know about the nitty-gritty of the hepatitis C lifecycle, there are still many gaps in our knowledge, including understanding in more explicit detail the structure and function of the membrane-bound replication complex. That's an area that Ralf and his lab continue to work on.

Other areas where more progress is needed is what you alluded to, Joao. What are the mechanisms involved in virus-associated pathology? How do those get going? How are they sustained over time? What is the actual link between hepatitis C and the development of liver cancer? Are there direct viral effects that are responsible, or is it just chronic inflammation,

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liver damage, and regeneration that raise the chances of someone developing cancer?

JM: What do you think, Ralf?

RB: Just to give you a simple example, nobody has ever really seen a virus particle assembling in a cell. How the virus is formed and how it is released out of the cell is, for the most part, a mystery. With respect to the pathogenesis, I fully agree. We are still limited because of limited patient samples and the lack of small animal models where we can recapitulate the pathology.

Also, with new drugs being available, there is a real chance to look in more detail at mechanisms of chronicity. For instance, you can monitor the reconstitution of the immune response in patients in real time as they respond to treatment. These new drugs are also excellent tools for molecular virology. In that respect, I think HCV could become kind of a role model to understand the mechanisms of how viruses drive chronic infection in humans.

JM: Can you tell more about how you first got involved with research on HCV and where that interest came from?

RB: I started out doing my PhD thesis here at the Center for Molecular Biology in Heidelberg, working on the hepatitis B virus. By the time I had finished my PhD, the first HCV molecular clone was published by Mike Houghton and his team in 1989. I was considering what would be the next step for me, not totally sure whether I would stay in academia or move to industry. I had the opportunity to join an academic environment in the industry at the Central Research Unit of Hoffman-La Roche AG Basel, where my mission was to set up a hepatitis C virus program. It was clear that this new virus was medically relevant and there was a strong economic interest behind it. We thought that we should invest in finding a viral protease, the reason being that, just around that time, the protease inhibitors for HIV had been developed, and so it was kind of ''logical'' to go search for the next protease inhibitor.

I moved therefore as a post-doc to Hoffman-La Roche to set up the HCV program. That meant first cloning what we thought to be full-length HCV genomes and then studying the genome organization, how the proteins are made, how they are processed, and back to the original mission, finding the viral protease, which indeed proved to be one of the main drug targets. This was, in a nutshell, my encounter with the HCV field. After around 3 years, I decided to move back to academia. My boss at the Central Research Unit of Hoffman-La Roche very generously allowed me to take what I had produced there for my own academic research back in Germany. This gave me a good head start to set up my own research group at the University of Mainz.

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CR: That's really quite an amazing gift. Not typical!

JM: Yes, I was about to say the same. What about you, Charlie?

CR: My interest in this virus came from a slightly different perspective. When the papers reporting the genome organization of this new non-A, non-B hepatitis virus were published in *Science*, this led to its grouping with a family of viruses that I had been interested in for several years, the Flaviviridae. As a post-doc at Caltech, I had been working on the prototype member of that group, yellow fever virus, and then moved to Washington University to start my lab in 1986. In 1989, when hepatitis C burst on the scene, I found myself with another member of the family but without really the kinds of tools that we had in our work with yellow fever virus. Steve Feinstone, who was working at the FDA and was interested in developing vaccines, was one of the pioneers in the study of hepatitis virus. Steve had seen a paper of ours reporting a functional molecular clone of the yellow fever virus 17D strain, a live-attenuated vaccine strain, and he was interested in seeing if the yellow fever vaccine strain could be engineered to express some hepatitis C virus proteins. He hoped that such a chimeric virus might serve as a live attenuated vaccine for both yellow fever and hepatitis C. We got to talking about the project and came to the conclusion that, although we had the sequence of a substantial chunk of the viral genome, including the polyprotein, we didn't know where the proteins started and stopped and how they were made. That really pushed us to begin fairly straightforward studies to help unravel that. Ralf and his group also worked on this, and there were several other groups in Japan, Italy, and elsewhere that were all working in parallel on defining the viral proteins using artificial expression systems since we didn't have an infection system that we could use. For me, our HCV work really began as a small side project that was started by myself and a very talented and energetic technician, Arash Grakoui, and then grew over time to take up an increasingly larger portion of my lab's efforts.

JM: Those were very different starts, I would say.

CR: Yeah. Things would have moved more quickly if we had the tools then that we have today.

JM: That's what I was about to ask. Do you recall a particular moment of struggle or a moment that things got really hard or that you felt stuck? A major roadblock?

CR: Ralf and I could go through the whole succession of triumphs and frustrations. I think that has been a hallmark of the HCV effort. Finding experimental tools to study the virus really

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took quite a while to establish. With each breakthrough, we also encountered barriers. On the heels of understanding polyprotein processing, viral proteases, and so on, there was a desire to follow these up with functional studies in the context of virus replication. I think that the labs were really all working hard to make functional viral cDNA clones and cell culture systems. We didn't have easy ways to test the infectivity of RNA transcripts from HCV cDNA clones even if we could make them in a test tube. As it turned out, there was actually a sequence missing at the 3′ end of the genome, which was not identified until 1995, by Kunitada Shimotohno's lab and Alexander Kolykhalov in our lab. This was a highly conserved important part of the HCV RNA genome, required, as we know now, for replication. Even having that, the question was how do you put together a cDNA template to make RNA transcripts that mimic the viral genome RNA and test them? In 1997, we finally managed to construct a cDNA clone that could be used as a template to synthesize functional HCV genome RNA, as demonstrated in the chimpanzee model. This, I thought, was the material that we needed to really crack the cell culture problem. It turned out that we could not get these transcripts to do anything in cell culture. Again, a breakthrough and then a roadblock. Maybe I'll let Ralf pick up the story from there.

RB: We had the same ups and downs. I still remember all the discussions I had with Charlie at the annual HCV meeting in Venice, circa 1998, about where we would go if all we could do was to produce recombinant viral proteins. How could we ever be able to prove that any of these proteins do anything relevant without a replication system? After that meeting, my group and I were driving home, and we had a really serious discussion of whether we would stop working on HCV because it was clear that we were very close to a dead end.

CR: The other problem, too, was trying to find an assay to detect viral replication against this very high background, given all the RNA that we were transfecting in the cells.

RB: Yes. Volker Lohmann, in my lab, spent a year trying to get a simple RT-PCR up and running. What we learned was simply that we will never be able to detect a low replication of HCV in cell culture by using those methods. Our last shot was to make replicons, where we introduced a luciferase reporter or a selectable marker. I still remember the days when Volker did the first transfections, putting in a replicon with a luciferase where the luciferase expression was a readout for RNA replication. He always found a signal 2-fold difference above background. We said, "2-fold, so what? We'll never be able to work with that." Then, we used selectable replicons to select the cells where this viral RNA is stably replicating. We further transfected roughly 10^7 cells, and what we ended up with were 20–40 cell clones that were resistant to the drug we had used for selection. A technician in the lab, who is very talented in propagating cells, picked those clones and established cell lines out of that and then Volker prepared the RNA to run a northern blot. We were very pessimistic, and we did not even dare to spend the money to buy the radioactivity to make the probe until we needed it for something else.

We had this blot in the drawer for a few weeks already when we bought the radioactivity for another assay. When Volker came out of the dark room with an X-ray film in his hand, saying, "There are bands on this northern blot," it was so unbelievable! I couldn't believe it since I'm always a pessimistic guy. And after 5 years having nothing, all of a sudden a signal on a northern blot, which is not a very sensitive method! We flipped this blot around in every direction. Whatever we did, it looked like we had something replicating in cell culture. From then on, I must say, it moved super-fast because we had already many assays established over the last couple of years. It took us a only a little while to convince ourselves that this was really a self-replicating HCV RNA, which then became the replicon system. That was really the starting point, and my people decided not to quit and to stay in the lab.

CR: Just in the nick of time.

RB: Just at the right time. If it would have taken half a year longer, probably I would have been alone. Maybe with a technician, but that's it. It was really super-tight to get this up and running, but then we were super-happy.

CR: Yes, more than 15 years after the discovery of the virus, right? It was really a long road, with many hurdles to overcome.

JM: I can tell! By the way, do you remember when you first met each other?

CR: Now you're testing my fading memory. My guess is it was probably one of the hepatitis C meetings. Ralf, do you remember?

RB: Yes, I remember. It was a meeting at the nice UCSD campus, where we were sitting during breakfast, talking about how to clone this stupid genome that doesn't replicate in cells, where you advised me I should go for a single, sickly looking *E. coli* colony. Perhaps you remember when we thought maybe the reason we couldn't get HCV properly cloned was that it was just unstable in *E. coli*?

CR: Well, you know, after my Flavivirus experience, I was damaged goods, I guess. Those plasmids are so toxic for *E. coli* that they still present challenges to work with even today.

RB: I think probably it was 1993 or so. One of the first HCV meetings, where we met at the university in San Diego. This is a day I still remember pretty well because we were stuck with the same issues.

CR: Yeah, so that's getting to be more than a couple of decades now.

JM: My last question is a nonscientific question. What do you like to do when you're not working or at the lab or in a conference or attending meetings or talking to editors on the phone?

CR: I don't think I have anything that falls into that category! I'm always running behind on all fronts!

RB: I would say it's about the same on my side, although whenever it's possible I take my running shoes and go out for an hour into the forest where I'm living. That's very refreshing, not only to the legs and the blood circulation, but also the brain. But, in fact, I also do see a bit of a risk that the problem is when you are chased over time with such an intense schedule, you forget about your hobbies. It might be that I have to rediscover my hobbies once I meet retirement stage. I don't think about this for now, but the little time I have, of course, is spent primarily with my family. My son is 12 years old, and my daughters are much older now, 29 and 24. But whenever I have time for my own, I try to use it to do some sports.

CR: Yes, I do like hiking and the mountains, I have to admit. So usually, toward the end of summer, we will spend a couple of weeks in Wyoming at a cabin in the Wind River Range, which is a bit of a change from Manhattan.