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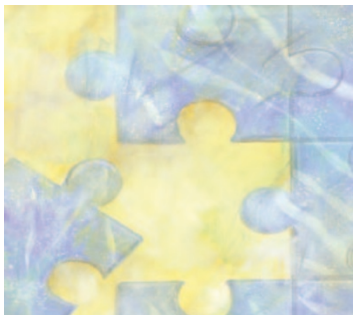
TECHNIQUES AND APPLICATIONS

Breakthrough for HCV research

Hepatitis C virus (HCV) afflicts more than 170 million people worldwide but until now HCV research has been severely hampered by the inability to produce infectious virus in cell culture. In a major breakthrough, three papers reporting the replication of full length HCV clones ***in vitro*** have just been published, paving the way for developing effective antiviral therapies and vaccines.

HCV primarily infects hepatocytes and causes hepatitis, cirrhosis of the liver and hepatocellular carcinoma. There is no vaccine and drug treatments are costly and have poor efficacy. The absence of a small-animal model and a cell-culture system for HCV have been obstacles to studying this virus, and researchers have relied on studying infections in humans and chimpanzees.

In the past five years, the development of ***in vitro*** HCV replicon systems has enabled viral molecular biology and virus–host interactions to be probed. Such systems use genomic and subgenomic clones that are transfected into hepatocyte cell lines. The main disadvantage of these systems is that the RNAs cannot replicate ***in vitro*** without acquiring adaptive mutations, nor do these systems produce infectious virions, so their relevance to the biology of wild-type infectious HCV isolates is questionable.



Three papers report the development of faithful ***in vitro*** replication systems for HCV. These studies built upon very recent advances: in the past two years, the Wakita group developed an ***in vitro*** system that replicates a subgenomic RNA that has not acquired any adaptive mutations, which formed the basis for the studies just published. All three studies used hepatocyte cell lines and importantly, all of the full-length replicons were either the JFH-1 HCV strain that was previously isolated from a fulminant-hepatitis patient by Wakita's group or a chimaera based on that strain. None of the full-length RNA clones that were used in these studies contained adaptive mutations, which is crucial, because the Bartenschlager group had shown that these mutations interfere with virus production and infectivity ***in vitro***. Therefore, the systems are representative of the wild-type HCV infection cycle. In all three studies, monitoring of viral RNA production by PCR, protein production by antibody labelling and classic dilution and infection studies were used to quantify RNA replication.

The different studies have common features. First, all of the ***in vitro*** systems replicate the full-length viral RNA and transfected cells produce virions — evidence of a complete virus life cycle. Second, viruses produced ***in vitro*** can be propagated efficiently using cell passage. Third, all three studies showed that the biophysical properties of the virions that are secreted by transfected cells are comparable to virions produced in chimpanzees infected with wild-type HCV. Finally, Wakita ***et al.***, in collaboration with the Liang group, used intravenous inoculation with ***in vitro***-produced virus suspensions to prove that the ***in vitro***-produced virus is infectious in chimpanzees. All three reports showed that antibodies against virus proteins neutralized the infectivity of virus that was produced ***in vitro***. Further, Wakita ***et al.*** and Zhong ***et al.*** blocked a putative cellular receptor, CD81, using anti-CD81 antibody, while Lindenbach ***et al.*** blocked the same receptor with a soluble recombinant CD81 fragment and prevented ***in vitro***-produced virus from infecting Huh-7.5 cells.

The development of these tissue-culture systems should accelerate the pace of hepatitis research, which is good news for those that have fallen prey to this global health scourge.

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References and links

ORIGINAL RESEARCH PAPERS Wakita, W. *et al.* Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nature Med.* 13 June 2005 (doi:10.1038/nm1268) | Lindenbach, B.D. *et al.* Complete replication of hepatitis C virus in cell culture. *Science* 09 June 2005 (doi:10.1126/science.1114016) | Zhong, J. *et al.* Robust hepatitis C virus infection *in vitro*. *Proc. Natl Acad. Sci. USA* 06 June 2005 (doi:10.1073/pnas.0503596102)