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News & Views

Hepatitis C: a successful story of cure

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On September 23, the 2016 Lasker-DeBakey clinical medical research award ceremony was celebrated in New York City. This annual prize has been awarded by the Lasker Foundation for over 71 years, and it is the most prestigious biomedical award in the United States, popularly known as "America's Nobel Prize". Indeed, 87 Lasker laureates have received the Nobel Prize, including 41 within the last 30 years (one example is the Chinese pharmaceutical chemist Youyou Tu, who won the 2011 Lasker-DeBakey award and four years later received the Nobel Prize). This year, the honor went to Ralf F. W. Bartenschlager (Heidelberg University, Germany), Charles M. Rice (Rockefeller University, NY, USA), and Michael J. Sofia (Arbutus Biopharma, PA, USA) for the development of cell culture system to study the replication of Hepatitis C virus (HCV) and for the use of this system to develop drugs capable of eliminating Hepatitis C.

In 1975, a disease associated with blood-transfusion was discovered and termed Non-A Non-B hepatitis (NANBH), but the causative agent for this type of hepatitis remained a mystery for more than a decade. In 1989, the laboratory of Michael Houghton at Chiron Corporation generated a lambda phage expression library using highly-infectious plasma from a NANBH chimpanzee. By screening with patient sera, one cDNA clone, 5-1-1, was identified as the viral agent responsible for NANBH which was then termed HCV [1]. It is an enveloped virus containing a positive-strand RNA genome belonging to the family of *Flaviviridae*. With approximately 9,600 nucleotides, the viral genome encodes a single polyprotein of more than 3,000 amino acids, which is cleaved into 10 viral proteins by viral and cellular proteases. Estimated 120 million individuals worldwide are currently infected by HCV, with the majority undiagnosed or untreated, and at the risk of developing cirrhosis, hepatocellular carcinoma or liver failure.

After the discovery of HCV, rapid progress had been achieved in the fields of diagnostics and epidemiology, but the viral RNA could not replicate in cell culture. It took 10 years to overcome this obstacle by sequential groundbreaking findings. The laboratory of Charles Rice and Kunitada Shimotohno identified a missing 3′-end in the reported HCV genome. The 3′-end fixed RNA transcripts were further corrected according to the consensus sequence of multiple independent cDNA isolates. Indeed, the resulting RNA transcripts after these improvements were found to be infectious in chimpanzees after intrahepatic injection by the laboratories of Charles Rice and Jens Bukh [2, 3]. Paradoxically, transfection of hepatic cell lines with this "validated" genomic RNA was not able to initiate any detectable viral replication. After a number of frustrating experiments, Volker Lohmann and his colleagues in the laboratory of Ralf Bartenschlager envisioned that different experimental approaches had to be taken. With the knowledge that structural proteins should be dispensable for RNA replication as shown by poliovirus, they replaced the gene fragments encoding HCV structure proteins with neomycin phosphotransferase and inserted an additional internal ribosome entry site (IRES) sequence to translate HCV non-structural proteins. When these RNA transcripts were transfected into a hepatoma cell line Huh7, few G418-resistant cell clones were obtained. Even more surprisingly, viral RNA with the right size could be detected by rather insensitive Northern-blot analysis [4]. With further evidence that the RNA was not a product of

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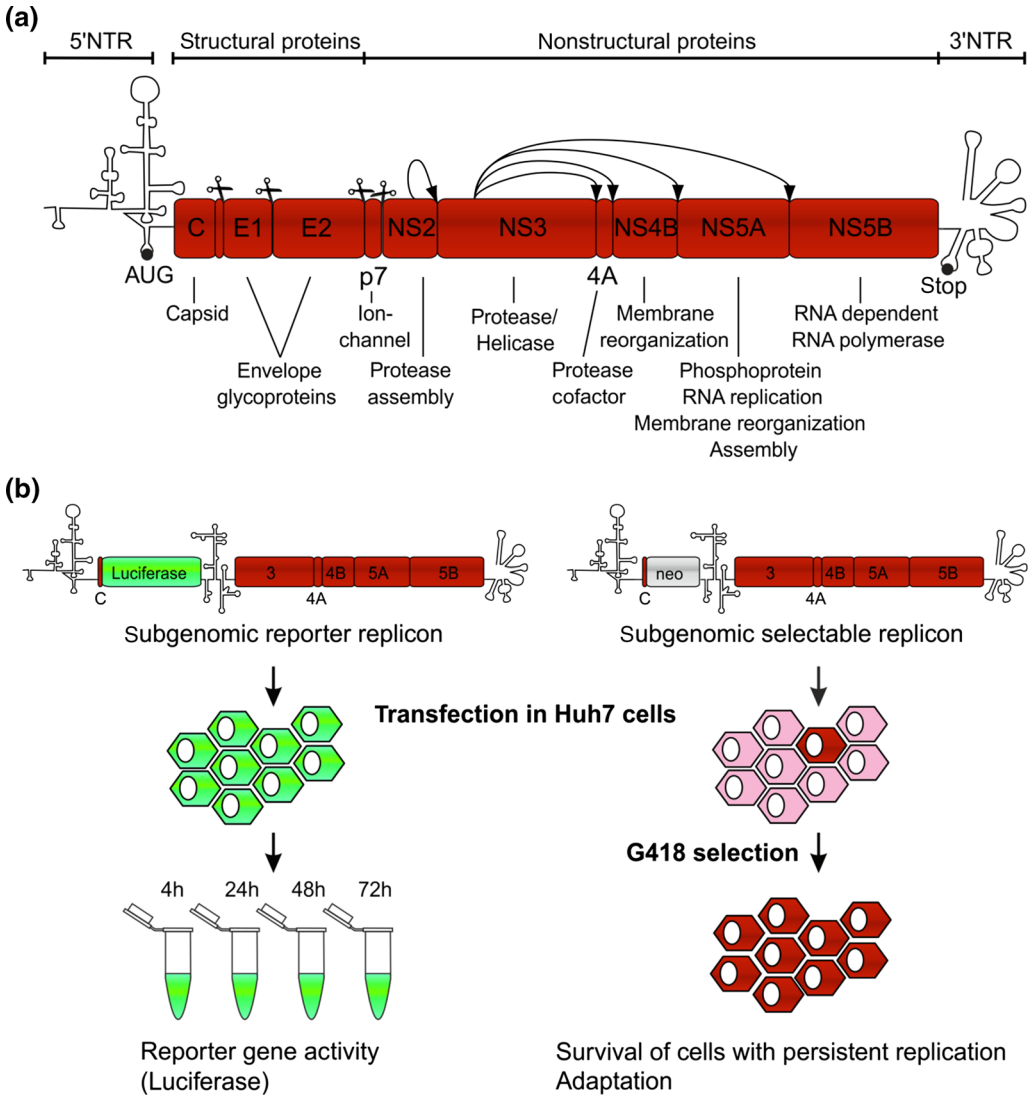

Fig. 1 (Color online) HCV genome organization and generation of replicon system. **a** HCV genome organization. The 5′ and 3′ nontranslated regions (NTRs) are indicated by their predicted secondary structures. Red boxes, coding regions; scissors, cleavages site by host proteases; arrows, cleavages site by viral proteases. **b** Basic organisation of HCV replicons. Subgenomic replicons contain the encephalomyocarditis virus IRES driving translation of the HCV nonstructural proteins and reporter genes (e.g., luciferase) or selection markers (e.g., neo). The in vitro RNA transcripts are transfected into hepatic cells such as Huh7. Left panel: The kinetic of viral replication is detected by reporter assays; right panel: selection for drug-resistant cell clones (e.g., by using G418 in the case of neo-containing replicons) allowing persistent replication of HCV replicon RNA *Source*: Dr. Volker Lohmann

unintended integration, they were convinced that the first robust and autonomous HCV replication was just established in a cell-based system. This replication system was named replicon and was soon reproduced by other laboratories. The replicon therefore was the first robust cell culture model for HCV and provided the urgently needed tool not only to study the basic aspects of viral replication, but also to develop specific antiviral drugs. The contributions of Charles Rice and Ralf Bartenschlager to the establishment of this groundbreaking HCV culture system and the importance of this model for the development of currently available therapies has now been honored by the Lasker award committee (Fig. 1).

The HCV RNA in the replicon seemingly evolved during the autonomous replication. After gain of one or several genetic mutations, viral replication in some replicon clones increased by up to several orders of magnitude [5]. When these cell culture adaptive mutations were introduced back to the full-length HCV construct, the lab of Ralf Bartenschlager established a transient replication assay and generated cell lines harboring full-length HCV replicons [6]. In addition to adaptive mutations within the

viral genome, the host cells further drastically contributed to the efficiency of RNA replication in the replicon system. In particular, after eliminating HCV RNA from replicon cells by interferon alpha treatment, a cell clone termed Huh7.5 was created which supported viral RNA replication much better than the naïve Huh7 cells [7]. Despite these improvements, the replicons were not able to produce infectious virus, even after re-introduction of the structural protein coding sequence. Moreover, the cell culture-adaptive RNA transcripts were not infectious when injected into chimpanzees. The next breakthrough in the field came from with the HCV isolate JFH-1, which was generated by Takaji Wakita's group from a fulminant hepatitis patient. It initiated exceptionally high levels of replication in hepatoma cells without requiring adaptive mutations. More importantly, infectious particles were secreted although at a rather low level [8]. The infectious particles in the culture medium dramatically increased when the JFH-1 RNA transfected cells were passaged for several weeks [9] or when a chimera of JFH-1 with another HCV strain J6 was used for transfection [10]. In both cases, the virus could efficiently spread through the cultivated cells. By then, the fully permissive and robust HCV cell culture system became available.

In the era before the discovery of hepatitis C, pilot daily treatment with interferon-alpha (IFN-α) was initiated in 10 patients in 1984. The exciting result was reported in 1986, that the serum aminotransferase level of all patients dropped down rapidly and one patient was eventually cured. In 1991, another trial of 10 patients with ribavirin, a broad spectrum antiviral nucleoside, showed that ALT levels of all patients decreased but returned to the pretreatment level 6 weeks post therapy [11]. However, combination of IFN-α with ribavirin achieved sustained virological response in 40% of patients [12]. Pegylated IFNs (Peg-IFN) were developed later to improve the half-life of IFN and to allow a more convenient weekly injection. Two first-generation HCV protease inhibitors telaprevir and boceprevir were approved by FDA in 2011 for combination with Peg-IFN and ribavirin. The IFN-based therapy remained the standard care until the 2010s. Nevertheless, in the early study it was already realized that IFN-α is poorly tolerated in Hepatitis C patients. Many patients had to reduce the dose of drug due to the adverse effects.

In 2005, Michael Sofia left Bristol-Myers Squibb and joined a small company called Pharmasset in Princeton, USA. At the time, Pharmasset had just published a paper describing a compound PSI-6130 inhibiting HCV in the replicon assay [13]. PSI-6130 is a cytidine nucleoside analog that targets HCV RNA polymerase by causing premature termination of RNA. The weakness of this initial compound was its modest potency and poor bioavailability. Michael Sofia came up with an idea to develop a prodrug of PSI-6130 for a better absorption after oral administration. This new prodrug RG-7128 showed lack of resistance and absence of adverse effects, but the activity was still modest. The understanding of the metabolism of PSI-6130 and comprehensive screens for prodrug analogs finally led to a uridine based compound, PSI-7851. The active isomer of PSI-7851 was selectively synthesized and known as PSI-7977, which was finally named sofosbuvir [14]. The clinical phase II trial of sofosbuvir in combination with IFN and ribavirin showed great efficacy. Moreover, a clinical trial termed "Electron" showed that the IFN-free combination of sofosbuvir with ribavirin led to a 100% cure rate in genotype 2 and 3 patients [15]. Combination of sofosbuvir with ledipasvir, an inhibitor of the HCV NS5A protein, provided above 95% cure rate in genotype 1 patient [16]. The regulatory approval of sofosbuvir as the first IFN-free treatment regimen makes sofosbuvir the backbone for HCV therapy in the future.

Looking back to the story of HCV from its discovery to cure, it is amazing to see how fast each milestone was achieved and how combined efforts in both basic scientific research and translational drug discovery allowed the development of efficient therapies. The unprecedented progresses in this field are truly remarkable. We are in the era that HCV is no longer a threat of life but a curable disease and are grateful to those people who made it happen.

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Conflict of interest The author declares no conflict of interest.

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