Hepatitis C virus–host cell interactions uncovered

Judith M. Gottwein* and Jens Bukh*†‡§

Department of Infectious Diseases, Copenhagen University Hospital, 2650 Hvidovre, Denmark; †Department of International Health, Immunology, and Microbiology, Faculty of Health Sciences, The Panum Institute, University of Copenhagen, 2200 Copenhagen, Denmark; and ‡Hepatitis Viruses Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

Insights into virus–host cell interactions as uncovered by Randall *et al.* (1) in a recent issue of PNAS further our understanding of the hepatitis C virus (HCV) life cycle, persistence, and pathogenesis and might lead to the identification of new therapeutic targets. HCV persistently infects 180 million individuals worldwide, causing chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. The only approved treatment, combination therapy with IFN-α and ribavirin, targets cellular pathways (2); however, a sustained virologic response is achieved only in approximately half of the patients treated. Therefore, there is a pressing need for the identification of novel drugs against hepatitis C. Although most research focuses on the development of HCV-specific antivirals, such as protease and polymerase inhibitors (3), cellular targets could be pursued and might allow the development of broad-spectrum antivirals (2).

Because of the lack of HCV cell culture models, initial studies on HCV–host cell interactions used expressed or purified proteins. The impact of host cell proteins on HCV replication and entry could be studied after the development of the HCV replicon system (reviewed in ref. 1; see also ref. 4) and pseudoviral particles (5), respectively. Both systems were originally developed in the human hepatocellular carcinoma cell line Huh7. Randall *et al.* (1) investigated for the first time the biological relevance of a panel of putative cellular HCV interacting factors, identified in the described experimental systems and the current study, for the complete viral life cycle using a recently developed HCV genotype 2a (J6/JFH1) cell culture system (6), which uses the Huh7-derived highly permissible cell line Huh7.5 (7). After silencing of individual cellular proteins by using experimental RNA interference (RNAi) (Fig. 1*A*), Huh7.5 cells were infected with J6/JFH1, and the resulting HCV infectious titers were compared (1). Silencing of a total of 26 cellular host proteins modulated production of infectious HCV by at least 3-fold; silencing of 25 of these proteins decreased HCV infectious titers by as much as 42-fold, indicating their supportive role for HCV replication, assembly, release, or viral entry. Although the
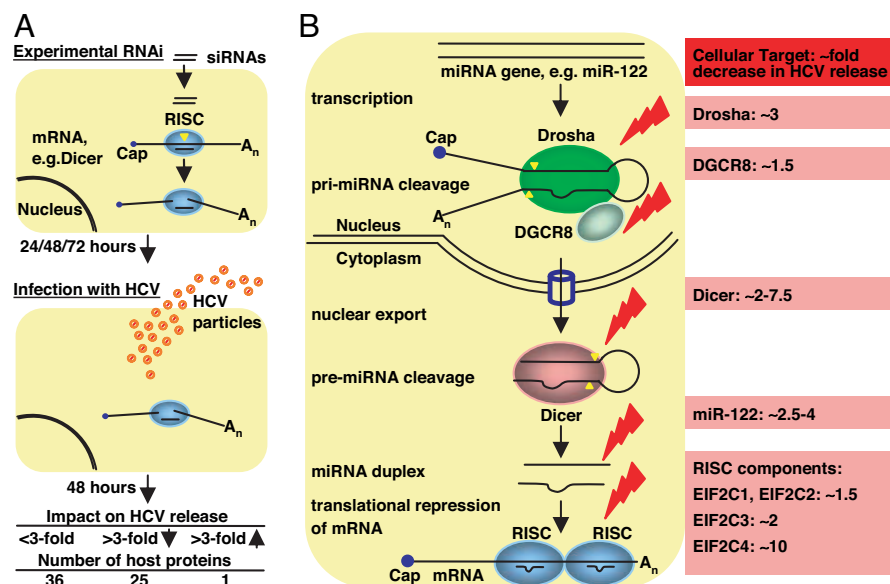

Fig. 1. Impact of host cell factors on production of infectious HCV particles: study design and results reported by Randall *et al.* (1). (*A*) Silencing of 62 host cell proteins by experimental RNAi. Small interfering RNAs (siRNAs) were transfected into Huh7.5 cells. Target mRNA is cleaved by cellular RNA-induced silencing complex RISC due to perfect sequence match to bound siRNA (9). Huh7.5 cells were infected with J6/JFH1 HCV viruses at 24, 48, or 72 h after transfection. HCV infectious titers in cell culture supernatants and intracellular HCV RNA levels were determined 48 h after infection. (*B*) Silencing of seven components of the miRNA biogenesis/RNAi pathway by experimental RNAi and depletion of miR-122. miRNAs such as miR-122 are transcribed as primary miRNAs (pri-miRNAs) and cleaved by Drosha (cofactor DGCR-8) in the nucleus. After nuclear export, the resulting pre-miRNA is processed by Dicer to form an miRNA duplex. Mature single-stranded miRNAs commonly bind their target mRNAs at several sites close to their 3′ end with imperfect match, resulting in assembly of RISCs and translational repression (14). HCV RNA contains two putative miR-122 binding sites in the untranslated regions (15); however, the mode and implications of miR-122 binding are unclear. Components targeted by Randall *et al.* are indicated by red flashes. Red boxes show the approximate resulting decrease in HCV infectious titers.

antiviral effect of silencing HCV interaction partners appeared to be moderate compared with direct silencing of HCV (>230-fold decrease in infectious titer), targeting of the same interaction partners by, e.g., small-molecule inhibitors or specific antibodies might result in increased antiviral efficacy. Thus, antibody-mediated blocking of the HCV coreceptor CD81 was shown to prevent infection with HCV (8) compared with an only 11-fold decrease in infectious titer after silencing (1). Such differences could be explained by the fact that in the experimental setup used (1), host protein expression is influenced not only by silencing efficacy but also by translation levels and turnover of these proteins, and possibly by regulation caused by HCV infection. On the other hand, moderate impacts on the HCV infec-

tious titer after silencing of presumably important HCV interacting partners, such as the cellular peptidases involved in HCV processing (SEC11L1 and HM13), could be caused by redundancy of cellular proteins used during the HCV life cycle. Such phenomena could also explain why 36 putative HCV interacting partners did not substantially influence the release of infectious HCV particles.

Excitingly, Randall *et al.* (1) discovered that the RNAi machinery exerted

© 2007 by The National Academy of Sciences of the USA

a proviral effect on the HCV life cycle because silencing of Dicer and components of the RNA-induced silencing complex RISC (Argonaute proteins EIF2C1–4) decreased HCV infectious titers (Fig. 1B). This adds strong evidence to the hypothesis that mammals, in contrast to plants and invertebrates, might not use RNAi as an innate antiviral defense mechanism (9–11). Previous findings supporting this hypothesis, specifically the absence of siRNAs and RNAi inhibitors during viral infections of mammalian cells (12), were also confirmed in the work of Randall et al. A likely explanation for this proviral effect of RNAi components could be their use in microRNA (miRNA) biogenesis (Fig. 1B) (11, 13, 14), given the demonstrated dependence of HCV on the liver-specific microRNA miR-122 (1, 15). This hypothesis is supported by the finding that silencing of Drosha or DCRG-8, specific for miRNA biogenesis (Fig. 1B), resulted in a similar decrease in the production of infectious HCV particles as silencing of the downstream components common for miRNA biogenesis and RNAi (1).

miRNAs are conserved 21- to 23-nt noncoding RNA molecules regulating gene expression at the posttranscription level in plants and animals (14). Their sheer abundance suggests that interactions between viruses and miRNAs might have evolved. Particularly, herpes viruses express miRNAs to regulate their own and possibly also host gene expression (11, 12). As an RNA virus with a cytoplasmatic life cycle, HCV apparently does not encode miRNAs

There is a pressing need for identification of drugs against hepatitis C.

(1, 12); however, HCV is so far the only virus for which a dependence on an endogenous miRNA has been demonstrated (1, 11, 15). While a direct interaction of miR-122 with the 5′ UTR of HCV seems to be important for HCV genotype 1 replication (15), Randall et al. (1) show the importance of miR-122 for the entire viral life cycle of genotype 2 (Fig. 1B). The mechanism of this interaction remains unknown. Alternatively to a direct role for HCV replication, miR-122 could be sequestered from its targets by physical interaction with HCV genomes; indeed, HCV replication has been shown to increase the expression of genes involved in cholesterol biosynthesis, which are modulated by miR-122 and thought to be of importance for the HCV life cycle (reviewed in ref. 1).

Liver-specific factors necessary for the HCV life cycle could be determinants of HCV liver tropism. By detailed miRNA profiling, Randall et al. (1) show the liver-specific miR-122 to be the most predominant miRNA in human liver and in cultured Huh7.5 cells, as previously suggested by studies of human, rat, and mouse liver and Huh7 cells (15–17). Facing the differences observed between miRNA profiles of human liver and Huh7.5 cells (1), it should be emphasized that the currently available HCV cell culture models use a tumor cell line differing from normal hepatocytes. Thus, the biological relevance of results generated in these systems should ultimately be verified in primary hepatocytes and in vivo. Interestingly, analysis of Huh7.5 cells containing an HCV genotype 1b replicon showed that HCV replication differentially regulated the cellular miRNA environment (1), as already demonstrated for the mRNA and protein environment in replicon-containing cells and in vivo (18–23). Microarray and proteomics studies of HCV-infected Huh7.5 cells would complete the picture of this aspect of complex virus–host interactions. Finally, as soon as cell culture systems for the six major HCV genotypes become available, it would be interesting to study genotype-specific interaction with host cell proteins.

1. Randall G, Panis M, Cooper JD, Tellinghuisen TL, Sukhodolets KE, Pfeffer S, Landthaler M, Landgraf P, Kan S, Lindenbach BD, et al. (2007) Proc Natl Acad Sci USA 104:12884–12889.
2. He Y, Duan W, Tan SL (2007) Drug Discovery Today 12:209–217.
3. Pawlotsky JM, Chevaliez S, McHutchison JG (2007) Gastroenterology 132:1979–1998.
4. Ng TI, Mo H, Pilot-Matias T, He Y, Koev G, Krishnan P, Mondal R, Pithawalla R, He W, Dekhtyar T, et al. (2007) Hepatology 45:1413–1421.
5. Bartosch B, Vitelli A, Granier C, Goujon C, Dubuisson J, Pascale S, Scarselli E, Cortese R, Nicosia A, Cosset FL (2003) J Biol Chem 278:41624–41630.
6. Lindenbach BD, Evans MJ, Syder AJ, Wolk B, Tellinghuisen TL, Liu CC, Maruyama T, Hynes RO, Burton DR, McKeating JA, et al. (2005) Science 309:623–626.
7. Blight KJ, McKeating JA, Rice CM (2002) J Virol 76:13001–13014.
8. Wakita T, Pietschmann T, Kato T, Date T, Miyamoto M, Zhao Z, Murthy K, Habermann A, Krausslich HG, Mizokami M, et al. (2005) Nat Med 11:791–796.
9. Cullen BR (2002) Nat Immunol 3:597–599.
10. Voinnet O (2005) Nat Rev Genet 6:206–220.
11. Cullen BR (2006) Nat Genet 38(Suppl):S25–S30.
12. Pfeffer S, Sewer A, Lagos-Quintana M, Sheridan R, Sander C, Grasser FA, van Dyk LF, Ho CK, Shuman S, Chien M, et al. (2005) Nat Methods 2:269–276.
13. Cullen BR (2006) Nat Immunol 7:563–567.
14. Bartel DP (2004) Cell 116:281–297.
15. Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P (2005) Science 309:1577–1581.
16. Chang J, Nicolas E, Marks D, Sander C, Lerro A, Buendia MA, Xu C, Mason WS, Moloshok T, Bort R, et al. (2004) RNA Biol 1:106–113.
17. Lagos-Quintana M, Rauhut R, Yalcin A, Meyer J, Lendeckel W, Tuschl T (2002) Curr Biol 12:735–739.
18. Fang C, Yi Z, Liu F, Lan S, Wang J, Lu H, Yang P, Yuan Z (2006) Proteomics 6:519–527.
19. Jacobs JM, Diamond DL, Chan EY, Gritsenko MA, Qian W, Stastna M, Baas T, Camp DG, Carithers RL, Jr, Smith RD, et al. (2005) J Virol 79:7558–7569.
20. Abe K, Ikeda M, Dansako H, Naka K, Shimotohno K, Kato N (2005) Virus Res 107:73–81.
21. Bigger CB, Brasky KM, Lanford RE (2001) J Virol 75:7059–7066.
22. Bigger CB, Guerra B, Brasky KM, Hubbard G, Beard MR, Luxon BA, Lemon SM, Lanford RE (2004) J Virol 78:13779–13792.
23. Su AI, Pezacki JP, Wodicka L, Brideau AD, Supekova L, Thimme R, Wieland S, Bukh J, Purcell RH, Schultz PG, et al. (2002) Proc Natl Acad Sci USA 99:15669–15674.