Modulation of Hepatitis C Virus RNA Abundance by a

Catherine L. Jopling,[1] MinKyung Yi,[2] Alissa M. Lancaster,[1] Stanley M. Lemon,[2] Peter Sarnow[1]*

MicroRNAs are small RNA molecules that regulate messenger RNA (mRNA) expression. MicroRNA 122 (miR-122) is specifically expressed and highly abundant in the human liver. We show that the sequestration of miR-122 in liver cells results in marked loss of autonomously replicating hepatitis C viral RNAs. A genetic interaction between miR-122 and the 5′ noncoding region of the viral genome was revealed by mutational analyses of the predicted microRNA binding site and ectopic expression of miR-122 molecules containing compensatory mutations. Studies with replication-defective RNAs suggested that miR-122 did not detectably affect mRNA translation or RNA stability. Therefore, miR-122 is likely to facilitate replication of the viral RNA, suggesting that miR-122 may present a target for antiviral intervention.

MicroRNAs (miRNAs) are a class of small RNA molecules, ~21 to 22 nucleotides (nt) in length, that have been detected in many plant and animal species (*1*). Even certain animal viral RNA genomes encode miRNAs (*2*–*4*). Cloning efforts and computational predictions have indicated that there are ~800 miRNA-encoding genes in humans (*5*), which together regulate more than 5300 genes (*6*, *7*). Interaction of miRNAs with target mRNAs results in mRNA cleavage if the bound miRNA engages in perfect base complementarity with its target (*8*, *9*). However, in a few cases, imperfect base complementarity between a within the 3′ noncoding region (NCR) of the target mRNA.

Certain miRNAs are expressed ubiquitously, whereas others are expressed in a highly tissue-specific manner (*16*, *17*). MiR-122 is specifically expressed in the liver, where it constitutes 70% of the total miRNA population (*16*, *18*). To examine the role of miR-122 in regulating mRNA function, we first monitored the expression of miR-122 in liver tissue and liver cell lines. MiR-122 was detected in mouse and human liver, in cultured human Huh7 and mouse Hepa 1-6 liver cells, but not in human cervical carcinoma-derived HeLa

[1]Department of Microbiology and Immunology, Stanford University, Stanford, CA 94305, USA. [2]Center for Hepatitis Research, Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, TX 77555–1019, USA.

*To whom correspondence should be addressed. E-mail: psarnow@stanford.edu

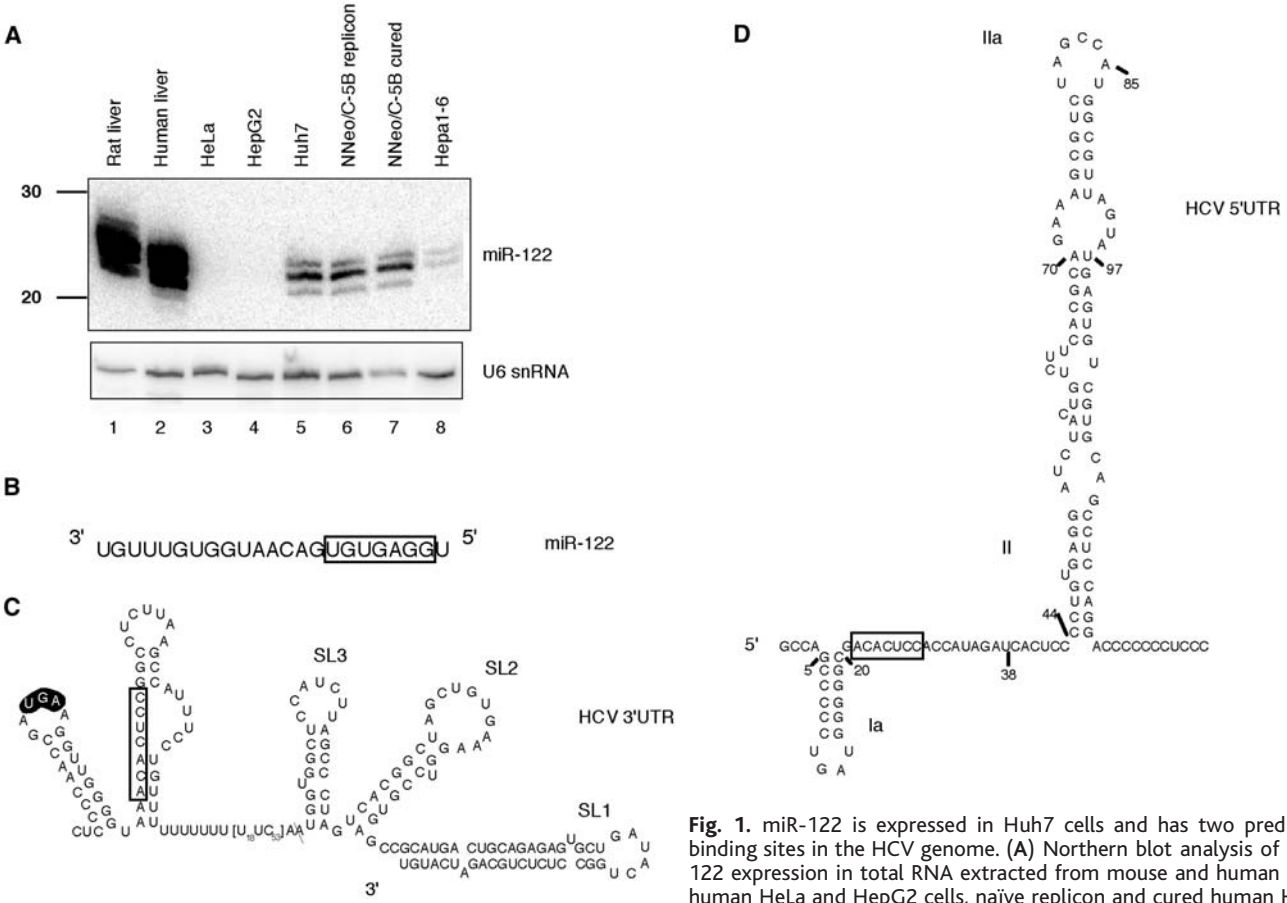

Fig. 1. miR-122 is expressed in Huh7 cells and has two predicted binding sites in the HCV genome. (**A**) Northern blot analysis of miR-122 expression in total RNA extracted from mouse and human liver, human HeLa and HepG2 cells, naïve replicon and cured human Huh7 cells, and mouse Hepa1-6 cells. Expression of U6 small nuclear (sn) RNA was used as a loading control. (**B**) Sequence of miR-122 with the seed sequences surrounded by a box. (**C** and **D**) Secondary structure of the (C) 3′ and (D) 5′ noncoding regions of the HCV genotype 1a strain H77c, with predicted miR-122 binding sites indicated. The seed matches are enclosed in boxes. SL, stem-loop; UTR, untranslated region.

cells or even in human liver-derived HepG2 cells (Fig. 1A).

Hepatitis C virus (HCV) is a hepatotropic, positive-stranded RNA virus belonging to the family *Flaviviridae*; it is a major cause for chronic liver disease with an estimated 170 million people infected (*19*). Although both Huh7 and HepG2 cells are derived from human hepatocytes, HCV RNA constructs can only replicate in Huh7 cells. To explore whether this could be related to the presence of miR-122 in permissive Huh7 cells, we inspected the 9600-nt, positive-strand, viral RNA genome for potential miR-122 binding sites that fulfill the rules for a successful miRNA-target mRNA interaction. We searched for sequences in the viral mRNA that could engage in Watson and Crick base pairing with nucleotides 2 through 8, the "seed sequence" of miR-122 (*6*, *20*), and we noted two predicted binding sites for miR-122 (Fig. 1B) in the viral NCRs. One is located within the viral 3′ NCR of the genotype 1a (Fig. 1C). Although this sequence is in the "variable region" of the 3′ NCR, the seed match sequence itself is highly conserved among the six HCV genotypes (table S1). The second miR-122 binding site is also conserved and is located within the 5′ NCR, only 21 nt from the 5′ end of the viral genome (Fig. 1D). With the exception of genotype 2, the putative seed match sequence is flanked by adenosines (table S1), indicative of a high confidence miRNA-binding site (*6*).

To determine whether miR-122 regulates HCV gene expression, we tested whether its inactivation would alter the abundance of an autonomously replicating, dicistronic HCV RNA replicon. Huh7 cells stably expressing the genotype 1b strain HCV-N replicon NNeo/C-5B were used (Fig. 2A) (*21*). To inactivate miR-122 in this cell line, NNeo/C-5B cells were transfected with a 2′-O-methylated RNA oligonucleotide (122-2′OMe) with exact complementarity to miR-122. Such oligonucleotides have been shown to sequester miRNAs (*22*, *23*). As a control for functional inactivation of miR-122, we monitored the expression of enhanced green fluorescent protein (eGFP) sensor mRNAs that contained sequences complementary to miR-122 in their 3′ NCR (eGFP-122). Because of its complete complementarity, miR-122 should lead to the nucleolytic degradation of the eGFP mRNAs. Indeed, little full-length eGFP-122 RNA was detected in cells transfected with plasmids encoding eGFP-122 (Fig. 2A, lane 3), although a similar RNA that contained sites complementary to the brain-specific miR-124 was expressed at high levels (Fig. 2A, lane 2). Upon transfection with 122-2′OMe, the amount of eGFP-122 RNA markedly increased (Fig. 2A, lane 4), whereas a randomized oligomer (Rand-2′OMe, lane 5) and an oligomer complementary to miRNA

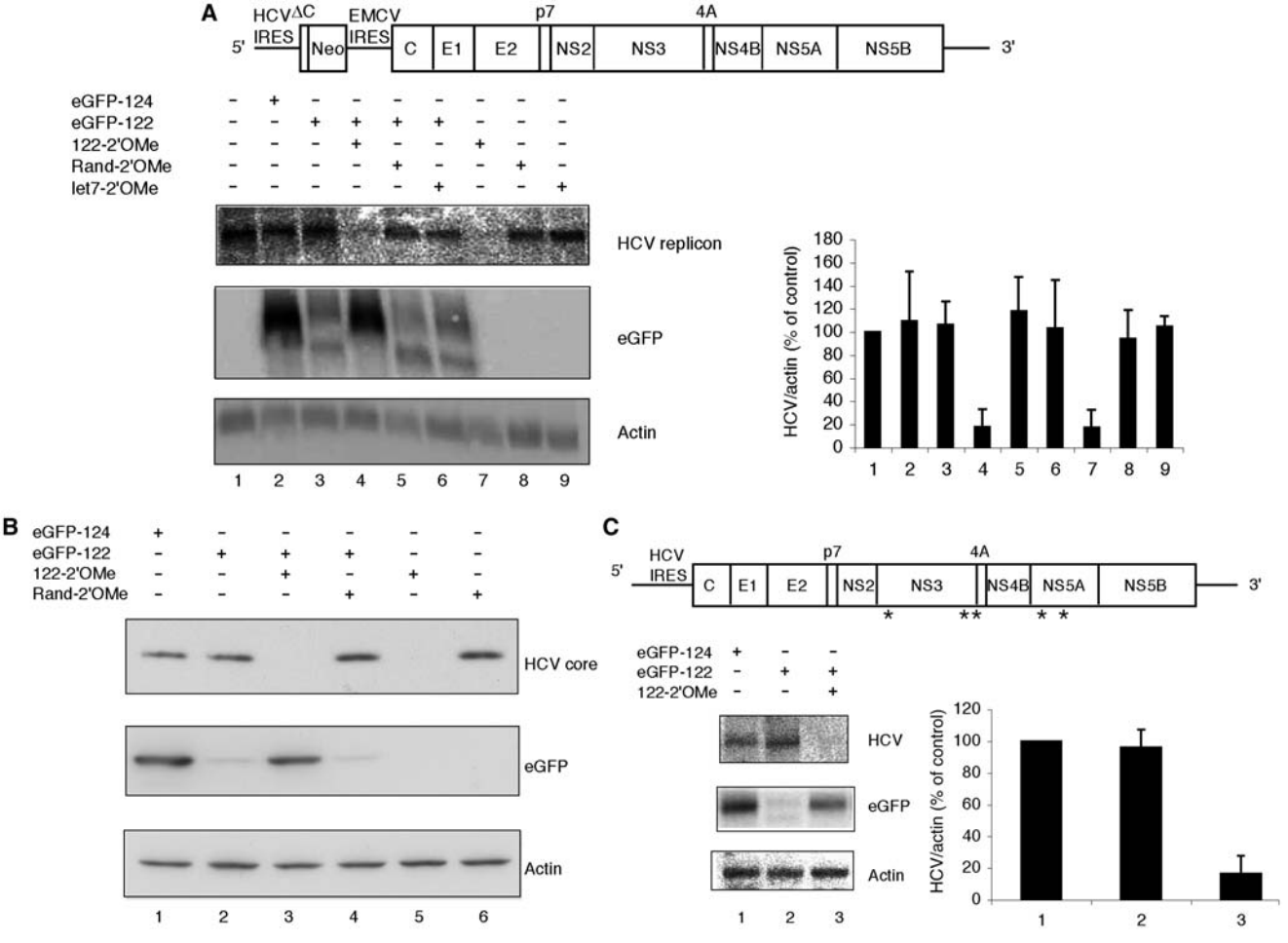

Fig. 2. Sequestration of miR-122 reduces HCV RNA and protein abundance in replicon cells. (**A**) Northern blot analysis of HCV, eGFP, and actin RNA in the NNeo/C-5B replicon cell line. In these replicon RNAs (*21*), the HCV internal ribosome entry site (IRES) directs the synthesis of the neomycin resistance gene product, and the encephalomyocarditis viral IRES (EMCV) directs the synthesis of the structural and nonstructural (NS) proteins of the HCV-N 1b strain. The eGFP sensor plasmids and 2′-O-methylated oligonucleotides were introduced into cells by lipofectamine 2000–mediated transfection, and total RNA was extracted 48 hours later. Quantitation of the HCV-actin mRNA ratios from three independent Northern blot experiments and the standard deviations are shown. (**B**) Western blot showing levels of HCV core protein, eGFP, and actin 48 hours post-transfection with the indicated eGFP sensor plasmids and 2′-O-methylated oligomers. (**C**) Northern analysis of HCV, eGFP, and actin RNA in Huh7 cells containing the genome-length genotype 1a H77c RNA and transfected with eGFP-122 and 122-2′OMe. Quantitation of the HCV-actin mRNA ratios from three independent Northern blot experiments and the standard deviations are shown. The locations of cell culture–acquired adaptive mutations in the viral RNA are indicated by asterisks in the top diagram.

let-7a (let7-2′OMe) had no effect (Fig. 2A, lane 6). The level of HCV viral replicon RNA was specifically reduced by ~80% when miR-122 was inactivated (Fig. 2A, lanes 4 and 7). Reduced mRNA abundance resulted in a decrease in HCV protein expression in cells transfected with the 122-2′OMe oligomer, whereas the level of eGFP protein increased under this experimental condition (Fig. 2B, lane 3).

To determine whether miR-122 would similarly affect RNA accumulation in cells newly transfected with replication-competent HCV RNA, RNA transcripts were synthesized from a cDNA that encodes the full-length genotype 1a strain H77c genome. Five adaptive mutations in the cDNA (Fig. 2C, top) promote efficient RNA replication in Huh7 cells (*24*). Introduction of these RNAs into Huh7 cells led to accumulation of viral RNA in the presence of endogenous miR-122 (Fig. 2C, lanes 1 and 2); in contrast, viral RNA failed to accumulate when miR-122 was sequestered by 122-2′OMe oligomers (Fig. 2C, lane 3). Furthermore, the 122-2′OMe oligomer did not affect total protein synthesis in transfected cells, excluding the possibility that the 122-2′OMe oligomer induced antiviral effects (fig. S1). Thus, miR-122 is required to maintain the abundance of both genotypes 1a and 1b RNA, both in a stable cell line supporting autonomous replication of a dicistronic replicon and upon direct transfection of minimally modified genomic RNA.

To investigate whether the putative miR-122 binding sites are required for the miR-122 effects on RNA abundance, we introduced mutations into the H77c cDNA. Transfection of H77c RNAs containing a 4-nt substitution mutation, m3′, in the predicted seed match in the 3′ NCR (Fig. 3A, left) did not diminish RNA accumulation (Fig. 3B, lane 2). In contrast, RNAs that contained single-nucleotide substitution mutations at the p3 and p6 positions in the seed match in the 5′ NCR or double mutations at the p3-p4 position failed to accumulate (Fig. 3B, lanes 4, 9, and 11). However, RNAs with mutations at p1 accumulated to similar levels (Fig. 3B, lane 8) as wild-type RNA (lane 6), supporting the idea that p1 does not contribute to formation of microRNA-mRNA complexes (*6*). These findings suggest that failure to recruit miR-122 to the HCV 5′ NCR caused loss of viral RNA or that the mutations affected translation, stability, or replication of HCV RNA.

If mutations in the HCV 5′ NCR reduced RNA accumulation because of poor binding of miR-122, ectopic expression of miR-122 RNAs that contain base complementary mutations should restore mutant microRNA–mutant RNA complexes. Ectopic expression of wild-type miR-122 RNAs did not rescue p3, p6, or p3-4 mutated viral RNAs (Fig. 3B, lanes 4, 9, and 11) but did enhance the abundance of wild-type viral RNAs (lane 7) and replicon RNAs (fig. S2). This demonstrated that the introduced miR-122 RNAs entered the cellular machinery as functional miRNA molecules and that the

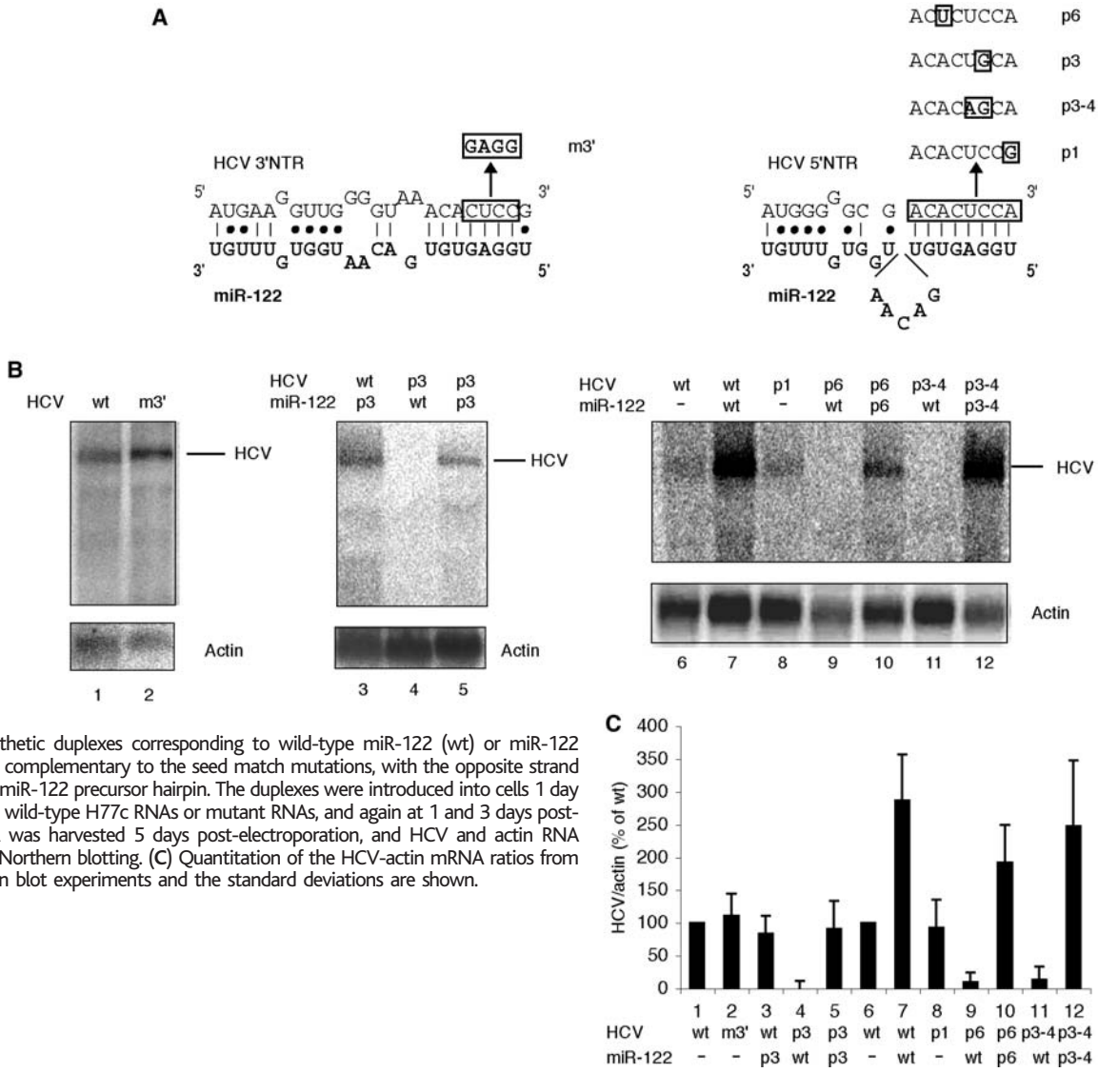

Fig. 3. The predicted miR-122 binding site in the 5′ noncoding region of HCV is required for viral RNA maintenance and directly interacts with miR-122. (**A**) Position of the mutations introduced into the H77c full-length RNA. The locations of a 4-nt substitution mutation in the seed match in the 3′ noncoding region (m3′) and single- or double-substitution mutations in the 5′ noncoding region seed match (p1, p3, p6, and p3-4) are shown. The mutated nucleotides are enclosed in boxes. (**B**) RNA was synthesized by in vitro transcription and introduced into Huh7 cells by electroporation, and HCV RNA levels were determined by Northern blotting 5 days later. Levels of actin mRNAs were determined as loading controls. Cells were transfected with synthetic duplexes corresponding to wild-type miR-122 (wt) or miR-122 with mutations in the seed complementary to the seed match mutations, with the opposite strand of the duplex based on the miR-122 precursor hairpin. The duplexes were introduced into cells 1 day before electroporation with wild-type H77c RNAs or mutant RNAs, and again at 1 and 3 days post-electroporation. Total RNA was harvested 5 days post-electroporation, and HCV and actin RNA levels were determined by Northern blotting. (**C**) Quantitation of the HCV-actin mRNA ratios from three independent Northern blot experiments and the standard deviations are shown.

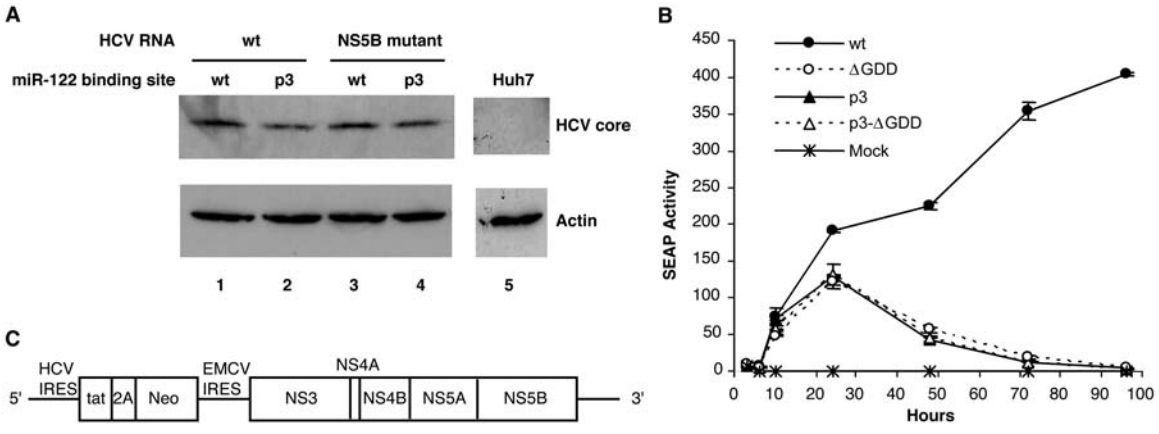

Fig. 4. Effects of mutation of the miR-122 binding site on mRNA translation and RNA stability. (**A**) Mutation of the miR-122 binding site does not affect HCV mRNA translation. The p3 mutation was introduced into a replication-deficient mutant of H77c, containing amino acid changes GDD to AAG at positions 2737 to 2739 in the viral polymerase NS5B (*24*). Lysates were harvested 20 hours after transfection of the wild-type and mutant RNAs, and HCV core protein and actin expression was determined by Western blotting. (**B**) Time course of SEAP production (activity displayed as arbitrary units) after transfection of En5-3 cells with various Ntat2ANeo replicon RNAs (*35*). The p3 mutation in the miR-122 binding site was introduced into both wild-type and the ΔGDD replication-deficient replicon. (**C**) The Ntat2ANeo replicon.

endogenous pool of miR-122 that mediates the accumulation of viral RNA is limiting. In contrast, expression of mutated p3, p6, and p3-4 miR-122 duplexes allowed accumulation of mutated viral RNAs (Fig. 3B, lanes 5, 10, and 12, and Fig. 3C), arguing for a genetic interaction between miR-122 and the 5′ NCR of the HCV genome. Furthermore, the rescue of mutated viral RNAs by miR-122 RNAs carrying complementary mutations provides evidence for a direct HCV RNA–miR-122 interaction, rather than an indirect effect through another miR-122 target.

It has been speculated that microRNAs reduce the accumulation of proteins encoded by target mRNAs by modulating translational efficiency of the mRNAs (*1*). Thus, we examined whether miR-122 modulates translation of HCV RNA, which occurs by an internal ribosome entry mechanism (*25*–*27*). We monitored the production of HCV core protein from transfected replicating and nonreplicating viral RNAs, containing or lacking miR-122 binding sites. Slightly greater amounts of core protein accumulated by 20 hours after transfection with wild-type (Fig. 4A, lane 1) versus p3-mutant RNA (Fig. 4A, lane 2). This difference is likely due to early replication of wild-type RNA. However, we were unable to detect full-length viral RNA by Northern analysis at this time point. To examine production of core protein from input RNAs in the absence of replication, we monitored translation of replication-defective viral RNAs. Both wild-type (Fig. 4A, lane 3) and p3-mutant (lane 4) RNAs containing a replication-lethal mutation in the viral RNA polymerase (GDD to AAG) produced similar amounts of core protein. These data may indicate that p3-mutant RNAs are less stable yet more efficiently translated than wild-type RNAs. An alternative interpretation suggests that mutant and wild-type RNAs display similar stabilities and translational efficiencies at 20 hours after transfection.

To distinguish between these two possibilities, we monitored the abundance of wild-type and mutated genotype 1b replicon RNAs derived from the strain HCV-N after their transfection into En5-3 cells. These replicon RNAs (Fig. 4C) express the human immunodeficiency virus tat protein and induce secretion of alkaline phosphatase (SEAP) in these cells in a manner that quantitatively reflects the intracellular abundance of the replicon RNAs (*28*). The mutant p3 replicon failed to accumulate over time after transfection compared to the wild-type replicon (Fig. 4B). Furthermore, the SEAP secretion profile of the p3 replicon mirrored that of cells transfected with the replication-deficient ΔGDD mutant, arguing that translation and stability of mutant p3-replicon RNAs are not affected by the p3 mutation (Fig. 4B). Taken together, these findings suggest that mutation of the seed match sequence for miR-122 in the HCV 5′ NCR does not primarily affect RNA translation or stability, at least at early times after RNA transfection, and likely affects viral RNA replication. In the absence of miR-122, core-encoding sequences have been reported to interact with nucleotides 24 to 38 in the viral 5′ NCR, resulting in translational inhibition in dicistronic mRNAs (*29*). However, mutations encompassing the miR-122 binding site have been shown to primarily affect replication of replicon RNAs (*30*) in cells expressing miR-122, suggesting that miR-122 may aid in RNA folding or RNA sequestration in replication complexes.

Although two plant miRNAs interact with the 5′ NCR of their target mRNAs (*31*), this has not been observed for animal miRNAs (*6*). Thus, our finding that the HCV genome recruits miR-122 to its 5′ end raises the question of whether 5′ NCRs in other viral or host cell mRNAs can also be targeted by microRNAs and whether such interactions regulate mRNA translation, mRNA turnover, or possibly RNA localization. HCV RNA can replicate in non-hepatic cells (*32*–*34*), raising the question of whether the role of miR-122 in regulating HCV gene expression is liver-specific. What are the natural targets for miR-122 in the liver, and is their expression affected by HCV infection? Finally, current therapies against HCV are frequently ineffective; thus, there is a need to search for alternative antiviral targets. Sequestration of host-encoded miR-122 could provide a possible antiviral tool against a rapidly evolving viral genome.

References and Notes

1. Y. Tomari, P. D. Zamore, *Genes Dev.* **19**, 517 (2005).
2. Y. Bennasser, S. Y. Le, M. L. Yeung, K. T. Jeang, *Retrovirology* **1**, 43 (2004).
3. S. Pfeffer *et al.*, *Science* **304**, 734 (2004).
4. C. S. Sullivan, A. T. Grundhoff, S. Tevethia, J. M. Pipas, D. Ganem, *Nature* **435**, 682 (2005).
5. I. Bentwich *et al.*, *Nat. Genet.* **37**, 766 (2005).
6. B. P. Lewis, C. B. Burge, D. P. Bartel, *Cell* **120**, 15 (2005).
7. X. Xie *et al.*, *Nature* **434**, 338 (2005).
8. G. Hutvagner, P. D. Zamore, *Science* **297**, 2056 (2002).
9. S. Yekta, I. H. Shih, D. P. Bartel, *Science* **304**, 594 (2004).
10. X. Chen, *Science* **303**, 2022 (2004).
11. J. G. Doench, C. P. Petersen, P. A. Sharp, *Genes Dev.* **17**, 438 (2003).
12. J. G. Doench, P. A. Sharp, *Genes Dev.* **18**, 504 (2004).
13. P. H. Olsen, V. Ambros, *Dev. Biol.* **216**, 671 (1999).
14. S. Saxena, Z. O. Jonsson, A. Dutta, *J. Biol. Chem.* **278**, 44312 (2003).
15. Y. Zeng, R. Yi, B. R. Cullen, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 9779 (2003).
16. M. Lagos-Quintana *et al.*, *Curr. Biol.* **12**, 735 (2002).
17. L. F. Sempere *et al.*, *Genome Biol.* **5**, R13 (2004).
18. J. Chang *et al.*, *RNA Biol.* **1**, 106 (2004).
19. J. H. Hoofnagle, *Hepatology* **36**, S21 (2002).
20. B. P. Lewis, I. H. Shih, M. W. Jones-Rhoades, D. P. Bartel, C. B. Burge, *Cell* **115**, 787 (2003).
21. M. Ikeda, M. Yi, K. Li, S. M. Lemon, *J. Virol.* **76**, 2997 (2002).
22. G. Hutvagner, M. J. Simard, C. C. Mello, P. D. Zamore, *PLoS Biol.* **2**, E98 (2004).
23. G. Meister, M. Landthaler, Y. Dorsett, T. Tuschl, *RNA* **10**, 544 (2004).
24. M. Yi, S. M. Lemon, *J. Virol.* **78**, 7904 (2004).
25. H. Ji, C. S. Fraser, Y. Yu, J. Leary, J. A. Doudna, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 16990 (2004).
26. G. A. Otto, J. D. Puglisi, *Cell* **119**, 369 (2004).
27. T. V. Pestova, I. N. Shatsky, S. P. Fletcher, R. J. Jackson, C. U. Hellen, *Genes Dev.* **12**, 67 (1998).
28. M. Yi, F. Bodola, S. M. Lemon, *Virology* **304**, 197 (2002).
29. Y. K. Kim, S. H. Lee, C. S. Kim, S. K. Seol, S. K. Jang, *RNA* **9**, 599 (2003).
30. P. Friebe, V. Lohmann, N. Krieger, R. Bartenschlager, *J. Virol.* **75**, 12047 (2001).
31. R. Sunkar, J. K. Zhu, *Plant Cell* **16**, 2001 (2004).

32. S. Ali, C. Pellerin, D. Lamarre, G. Kukolj, *J. Virol.* **78**, 491 (2004).
33. T. Kato *et al.*, *J. Virol.* **79**, 592 (2005).
34. Q. Zhu, J. T. Guo, C. Seeger, *J. Virol.* **77**, 9204 (2003).
35. Materials and methods are available as supporting material on *Science* Online.
36. We thank K. Kirkegaard for discussions and critical reading of the manuscript. Supported in parts by NIH grant nos. AI47365 and GM069007 (P.S.), AI40035 (S.M.L.), and AI63451 (MK.Y) and by International Research Fellowship 066592/Z/01/Z from the Wellcome Trust (C.L.J.).

Supporting Online Material
www.sciencemag.org/cgi/content/full/309/5740/1577/DC1
Materials and Methods
Figs. S1 and S2
Table S1

8 April 2005; accepted 8 August 2005
10.1126/science.1113329

Recombination Regulation by Transcription-Induced Cohesin Dissociation in rDNA Repeats

Takehiko Kobayashi[1,2]* and Austen R. D. Ganley[1]

Organisms maintain ribosomal RNA gene repeats (rDNA) at stable copy numbers by recombination; the loss of repeats results in gene amplification. Here we report a mechanism of amplification regulation. We show that amplification is dependent on transcription from a noncoding bidirectional promoter (E-pro) within the rDNA spacer. E-pro transcription stimulates the dissociation of cohesin, a DNA binding protein complex that suppresses sister-chromatid–based changes in rDNA copy number. This transcription is regulated by the silencing gene, *SIR2*, and by copy number. Transcription-induced cohesin dissociation may be a general mechanism of recombination regulation.

In most organisms, recombination is necessary for DNA repair, chromosome segregation, and the rescue of stalled replication forks. If not properly regulated, however, recombination can lead to genomic instability (*1*) and can be toxic to cells (*2*). It is not clear how cells maintain only the positive effects of recombination.

In repeated-gene families, such as the ribosomal RNA (rRNA) gene repeats (rDNA), recombination helps maintain copy number (*3*) and the evolutionary stability of the repeats (*4*). The number of rDNA copies is tightly regulated; if repeats are deleted or inserted, copy number is quickly restored to that of the wild type (*5*, *6*). One way that copy number is maintained is by gene amplification after deletional recombination. In the yeast *Saccharomyces cerevisiae,* this amplification is dependent on the replication-fork blocking protein, *FOB1*, and a ~520–base pair (bp) *cis*-acting factor called EXP, which is found in the rDNA intergenic spacer (IGS) (Fig. 1A) (*6*, *7*). In a recent *Saccharomyces* species phylogenetic footprinting study, we found a highly conserved sequence that corresponds to a previously identified bidirectional RNA polymerase II (pol II) promoter (*8*) in EXP (*9*). This EXP promoter (named E-pro) does not appear to be associated with any coding function, and its position and conservation suggested it might play a role in rDNA amplification.

To determine whether E-pro is involved in rDNA amplification, we replaced it with galactose-inducible pol II promoters (unidirectional *GAL7* and bidirectional *GAL1/10* promoters) (fig. S1) in an *S. cerevisiae* strain containing only two rDNA copies (two-copy strain), and we observed the effects on amplification. Reintroduction of a plasmid-borne *FOB1* gene into the two-copy strain stimulated rDNA amplification, and the resulting rDNA copy-number increase can be visualized by an increase in the size of chromosome XII (chr XII) by using pulsed-field gel electrophoresis [contour-clamped homogeneous electric field (CHEF)] (*7*). The deletion of E-pro abolished amplification ability (Fig. 1, B and C), and when E-pro was replaced with the *GAL7* promoter in either direction, amplification ability was not rescued. However, when E-pro was replaced with the bidirectional *GAL1/10* promoter (GAL1/10 strain), the introduction of *FOB1* resulted in amplification.

To confirm that amplification depends on E-pro transcription, we changed the carbon source from galactose to glucose to inhibit transcription in the GAL1/10 strain. The size of chr XII continued to increase in galactose-grown cells, but did not increase in glucose-grown cells over 150 generations (Fig. 1D). Furthermore, the chr XII bands of cells grown in glucose were sharp, indicating the inhibition of rDNA recombination (*6*). To investigate whether read-through transcription or another function of E-pro is required for amplification, we blocked each direction of GAL1/10 transcription by using a transcriptional terminator. Blockage in either direction resulted in the loss of amplification ability (Fig. 1D). Therefore, bidirectional E-pro transcription is essential for rDNA amplification.

How can transcription from E-pro trigger recombination and hence amplification? One way is through cohesin association. Cohesin is a multifunctional protein complex involved in chromatin structure (*10*), and its localization is inversely correlated with transcription, suggesting that transcription disrupts cohesin association (*11*, *12*). Cohesin association is thought to hold chromatids in place, leading to equal (versus unequal) sister-chromatid recombination and thereby preventing changes in copy number after the formation of double-strand breaks (DSBs) (*13*). Thus, E-pro transcription may result in cohesin dissociation, allowing a change in copy number. Chromatin immunoprecipitation (ChIP) assays were performed with a GAL1/10 strain carrying hemagglutinin (HA) epitope–tagged Mcd1p (a cohesin complex component) in conjunction with seven rDNA primer combinations (Fig. 2). In the wild-type strain grown in both glucose and galactose, the cohesin associating region (CAR) gave the strongest signal of cohesin association, as found previously (*13*, *14*), and the pattern in the galactose-grown GAL1/10 strain was similar. However, when grown in glucose, the GAL1/10 strain showed much stronger cohesin association throughout the IGS. Thus, the repression of E-pro transcription leads to increases in cohesin association on both sides of E-pro, not just in CAR. This increase is consistent with bidirectional transcription dissociating cohesin in both IGS1 and 2, and it suggests that unidirectional transcription leaves cohesin association on the opposite side, inhibiting unequal sister-chromatid recombination. We also tested the effect of a cohesin mutation, *smc1-2* (*15*), in the GAL1/10 strain, and we confirmed that the amplification rate was increased (fig. S2).

The silencing gene *SIR2* suppresses rDNA copy-number change through effects on cohesin association, because *SIR2* loss results in the loss of cohesin association in the IGS (*13*). *SIR2* represses pol II–transcribed genes integrated in the rDNA (*16*, *17*). We therefore speculated that *SIR2* regulates recombination by repressing E-pro transcription. To test this,

[1]National Institute for Basic Biology and [2]The Graduate University for Advanced Studies, SOKENDAI, School of Life Science, 38 Nishigonaka, Myodaijicho, Okazaki, 444-8585 Japan.

*To whom correspondence should be addressed. E-mail: koba@nibb.ac.jp

Modulation of Hepatitis C Virus RNA Abundance by a Liver-Specific MicroRNA

Catherine L. Jopling, MinKyung Yi, Alissa M. Lancaster, Stanley M. Lemon and Peter Sarnow

Science **309** (5740), 1577-1581.
DOI: 10.1126/science.1113329