Supplementary Information

Synthetic Antibody Binding to a Preorganized RNA Domain of Hepatitis C Virus Internal Ribosome Entry Site Inhibits Translation

Deepak Koirala¹, Anna Lewicka¹, Yelena Koldobskaya¹, Hao Huang¹, Joseph A. Piccirilli¹,².*

¹ Department of Biochemistry and Molecular Biology, The University of Chicago, Chicago, IL, 60637, USA. ² Department of Chemistry, The University of Chicago, Chicago, IL, 60637, USA.

* Corresponding author, email: jpicciri@uchicago.edu
Supplementary Note 1

Compared to the wild type IRES, the JIIIabc crystallization construct (68 nts, Fig. 1b) contained a U144G (all numbering refers to the genotype 1b according to Brown et al.¹ and Honda et al.²) mutation at the 5ꞌ-end for efficient *in vitro* transcription and a U248C mutation and a C149 insertion to stabilize the III* helical stem (Fig. 1b). The U248C mutation substitutes the G○U wobble pair with a canonical G-C base-pair and the C149 insertion forms a canonical base-pair with the unpaired G243 (see Supplementary Fig. 1 and 2). As in the previous construct, nucleotides 181 – 218 of the IIIb domain were removed and capped by a GAAA tetraloop.

Supplementary Note 2

Analysis using PDBePISA³ reveals that within the epitope (RNA)-paratope (Fab) interface involving Fab CDRs, the JIIIabc interactions with Fabs HCV2 and HCV3 bury a total of 635 Å² and 936 Å² of surface area, respectively. By comparison, the JIIIabc – HCV2 interface buries the least surface area (635 Å²) among characterized Fab – RNA complexes. The binding interface in Fab BL3-6 – hairpin epitope complex,⁴,⁵ Fab BRG – ssRNA complex,⁶ Fab HAVx – bulge epitope complex⁷ and Fab2 – P4P6⁸ complex bury 1102 Å², 928 Å², 1315 Å² and 1316 Å² area, respectively. For JIIIabc – HCV3 complex, the surface area buried at the Fab-RNA interface (936 Å²) approximates that of Fab BL3-6 – hairpin epitope complex and Fab BRG – ssRNA complex, and is significantly lower than Fab HAVx – bulge epitope and Fab2 – P4P6 complexes. For comparison, interactions at the Fab-protein interfaces bury on average 777 ± 135 Å² area.⁹

Within the epitope-paratope interfaces for both complexes, the specific interactions of Fab residues involve stacking, electrostatic, and hydrogen bonding (direct and water-mediated) contacts. For HCV2, beginning from the CDR L3, Y95 stacks on G233 and also interacts with C232 2ꞌ-OH via a water-mediated hydrogen bond (*Fig. 3f*). Residues S93 and Y94 contact G233, C232 and IIIa G163 through a network of direct and water-mediated hydrogen bonds (*Fig. 3f*). Likewise, two tyrosines (Y58 and Y60) and threonine (T61) from CDR H2 contact IIIa U164, A165 and IIIc C237 by direct and water-mediated hydrogen bonding interactions (*Fig. 3g*). Residues from CDR H3 mainly recognize nucleotides within IIIa, except for hydrogen bonding interactions between Y106 with IIIc G231 and G232 (*Figs. 3h, i*). Two arginines (R104 and R109) and a serine (S105) recognize the phosphodiester of IIIa A162, G163 and U164 through direct and water-mediated hydrogen bonds and electrostatic interactions (*Fig. 3i*). While Y106 stacks on U164, main-chain functional groups of Y106 and Y107 form hydrogen bonds with G163 2ꞌ-OH (*Fig. 3h*).
For HCV3, the side-chain functional group of a scaffold residue (Y50) near the CDR L1 makes a hydrogen bond with the phosphate backbone of IIIa G159 (Fig. 3j). Only two CDR L3 residues, Y92 and S93, interact with the RNA, forming hydrogen bonds to the Watson-Crick face of IIIc G233 through hydrogen bonds (Fig. 3k), in contrast to Fab HCV2 in which CDR L3 makes extensive stacking and hydrogen bonding interactions with both IIIa and IIIc nucleotides (Fig. 3f). The CDR H1 residues Y34 and S36 make hydrogen bonding interactions with the ribose sugars of IIIa G163 and U164 and the phosphate backbone of IIIc G233, respectively (Figs. 3l, m). The CDR H2 predominantly uses serines (S55, S57, S58 and S60) to recognize IIIc nucleotides C232, G233 and U234 through direct and water-mediated hydrogen bonding interactions (Fig. 3l). Additionally, Y62 forms a hydrogen bond with U54 phosphodiester (Fig. 3l). For comparison, CDR H2 in HCV2 uses only two tyrosines and a threonine for recognizing both IIIa (U164, A165) and IIIc (G233, C237) nucleotides (Fig. 3g). The CDR H3 residues heavily interact with four IIIc (G159, U160, G161 and G163) and three (C232, G233 and U234) nucleotides, through extensive sets of direct and water-mediated hydrogen bonding and electrostatic interactions (Figs. 3j, k, m). These interactions include the following: S102 with IIIc G233; R103 with IIIa G163, IIIc C164 and G233; Y104 and S105 with IIIc G233 and U234, respectively; R106 with IIIa G159, U160 and G161; and R109 with IIIa U160 and IIIc G233 (Figs. 3j, k, m). Although both HCV2 and HCV3 predominantly use CDR H3 for JIIIabc recognition, the contacts with HCV3 involve both IIIa and IIIc nucleotides whereas in HCV2, the majority of CDR H3 interactions involve IIIa nucleotides (Figs. 3h-k, m).
Supplementary Fig. 1 Overall schematic of the HCV genome. About 9.6 kb (+)-sense ssRNA genome consists of a single open reading frame (ORF) flanked by 5’ and 3’ UTRs\(^{10-12}\) (a). The 5’-UTR is highly conserved among HCV strains and clinical isolates\(^{13-15}\). It contains four domains (labelled I through IV) comprising highly organized secondary structures that include the IRES elements (b). The numbering and the sequence refer to the HCV strain genotype 1b according to Brown et al.\(^1\) and Honda et al.\(^2\). The AUG indicates the start codon, and the yellow dashed lines represent the interactions between the nucleotides forming a pseudoknot\(^{16}\). Dashed, red box highlights the junction formed between sub-domains IIIa, IIIb and IIIc (called junction IIIabc or JIIIabc), which interacts with the 40S ribosome through protein eS27 (interaction sites are indicated in green).\(^{17}\)
Supplementary Fig. 2 Previous crystallography and cryo-EM studies of the JIIIabc. (a) The 2.8-Å crystal structure (PDB code: 1KH6) of the JIIIabc reported by Kieft et al.\textsuperscript{18} and (b) a model of the same junction obtained from 3.9-Å resolution cryo-EM structure of HCV IRES – 40S ribosome complex (PDB code: 5A2Q) reported by Quade et al.\textsuperscript{17} The JIIIabc construct (c) was observed as a dimer (d) in the crystal\textsuperscript{18}, in contrast to a conformation of the same junction (b, e) observed in the 3.9-Å resolution cryo-EM structure of the full-length IRES\textsuperscript{17}. Interestingly, the crystal structure corresponding to the dimeric form of the JIIIabc contained the same junction nucleotides and flanking base pairs in the helices IIIa, IIIb and III* as the monomeric form (cryo-EM structure) but loop IIIc is engaged with the same loop of the neighboring molecule to form a helical stem (d).
**Supplementary Fig. 3** Phage display selection construct of the HCV IRES and its binding affinity with Fabs HCV2 and HCV3. (a) The RNA construct for phage display selection includes 333-nucleotide long IRES (nts 40 to 372, genotype 1b) with an additional 3’-overhang sequence (red, x-module) that hybridizes to a biotinylated DNA oligonucleotide (green, complementary DNA). (b) Residues from CDRs of the Fabs HCV2 and HCV3. (c) A representative plot of fraction bound as a function of varying concentration of Fabs HCV2 (black circles) and HCV3 (blue diamonds) for the HCV IRES (333-nt without x-module sequence) as accessed from filter binding assay in 10 mM Tris-HCl (pH 7.4), 10 mM MgCl₂ and 200 mM NaCl buffer at 23°C. The reported $K_d$s represent the values (average ± standard deviation) obtained from ≥ 3 independent measurements.
Supplementary Fig. 4 Binding affinity of isolated JIIIabc constructs against Fabs HCV2 and HCV3. (a) The sequences corresponding to the isolated JIIIabc constructs (highlighted with colors) in the context of the full-length HCV IRES. (b) The isolated JIIIabc construct similar to that crystallized previously by Kieft et al. in a dimeric form (see Supplementary Fig. 2) (c) Representative plots of fraction bound as a function of varying concentration of Fabs HCV2 (black circles) and HCV3 (blue in 10 mM Tris-HCl (pH 7.4), 10 mM MgCl$_2$ and 200 mM NaCl buffer at 23°C for the JIIIabc construct. The reported $K_d$s represent the values (average ± standard deviation) obtained from ≥ 3 independent measurements. (d) The JIIIabc crystallization construct in this study. The gray colored nucleotides in b and represents the mutations or insertions compared to the wild-type sequence (see colored region in a).
Supplementary Fig. 5 Binding of the JIIIabc deletion constructs to Fabs HCV2 and HCV3. Both Fabs, HCV2 and HCV3 bind with intact JIIIabc (see, Supplementary Fig. 4). However, the isolated IIIa (b), IIIc (c) and IIIb/IIIc (d) constructs did not bind to either Fab, indicating a requirement for the intact JIIIabc tertiary structure in Fab recognition.
Supplementary Fig. 6 Structural models of the JIIIabc in complex with Fabs HCV2 (a) and HCV3 (b) at 1.81-Å and 2.75-Å resolution, respectively. The structures of JIIIabc RNA corresponding to the JIIIabc – HCV2 and JIIIabc – HCV3 complexes are depicted in (c) and (d), respectively. Orange spheres depict water molecules, and the gray mesh represents the 2|F_o| - |F_c| electron density map at 1σ contour level and carve radius 1.8 Å.
Supplementary Fig. 7 Comparison of the structures of the JIIIabc – HCV2 and JIIIabc – HCV3 complexes. The JIIIabc – HCV2 (a) and JIIIabc – HCV3 (b) complexes crystallized in the C1 2 1 and P4₃ space group lattices, respectively. The JIIIabc – HCV2 crystal contained one and the JIIIabc – HCV3 crystal contained three Fab – RNA complexes per asymmetric unit. (c) Superposition of the JIIIabc structures corresponding to the JIIIabc – HCV2 complex (blue) and JIIIabc – HCV3 complex (yellow) crystal structures. These two RNA structures are almost identical except that in JIIIabc – HCV2, the first G nucleotide remains stacked within the helical stem and in in the JIIIabc – HCV3, it flips out of the helix to make crystal contacts with the neighboring Fab molecule. Additionally, G233 flips out of the IΙlc helical stack in both complexes but in opposite directions, and interacts extensively with Fab residues within the Fab-RNA binding interface.
**Supplementary Fig. 8** Crystal packing and RNA-RNA contacts. The RNA-RNA contacts observed in the crystals of JIIIabc – HCV2 (a) and JIIIabc – HCV3 (b) complexes. In the JIIIabc – HCV2 complex, two symmetry mate RNA molecules make two sets of tetra-loop – receptor interactions involving helical stem IIIa (colored green) and the IIIb GAAA tetra-loop (colored gray), whereas in JIIIabc – HCV3 complex, the RNA-RNA contacts occur between two RNA copies within the asymmetric unit via GAAA tetra-loop – tetra-loop interactions (gray colored loops within sub-domain IIIb).
Supplementary Fig. 9 Comparison of the crystal structure and the cryo-EM model of the JIIIabc – 40S ribosome complex. (a) The 3.9-Å resolution model of the JIIIabc obtained from of HCV IRES – 40S ribosome complex (PDB code: 5A2Q). In this complex, ribosomal protein eS27 interacts directly with the four-way junction of JIIIabc. (b) The 1.81-Å resolution structure of the same region of the JIIIabc in complex with Fab HCV2 (this study). Fab HCV2 (or HCV3) recognize the same region of the JIIIabc RNA; all three proteins interact with similar nucleotides of IIIa and IIIc sub-domains and use physiochemically similar sets of protein residues to do so (see Fig. 5 and Supplementary Fig. 10). Importantly, the four-way junctions in these complexes appear identical (d) despite some structural deviations flanking the junction.
Supplementary Fig. 10 Protein-RNA binding interfaces in JIIIabc – HCV2, JIIIabc – HCV3 and JIIIabc – eS27 complexes. a) IIIa and (b) IIIb sub-domain interactions with Fab HCV2 CDR surface. (c, d) Analogous figures corresponding to the HCV3 and (e, f) eS27 (PDB code: 5A2Q). The X, Y and Z in the molecular surface of eS27 represent arbitrary assignments for clarity of the protein-RNA interface.
Supplementary Fig. 11 Schematics representing interactions of specific JIIIabc nucleotides (circled) with particular amino acid residues from HCV2 (a), HCV3 (b), and eS27 (c; PDB code: 5A2Q)\textsuperscript{17} Hydrogen bonding and stacking interactions are indicated by $\textsuperscript{\square}$ and $\textsuperscript{\|}$, respectively. The red spheres in d and e represent water molecules.
Supplementary references


