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Distinct Regions of Human eIF3 Are Sufficient for Binding to the HCV IRES and the 40S Ribosomal Subunit

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Translation of the hepatitis C virus (HCV) genomic RNA initiates from an internal ribosome entry site (IRES) in its 5′ untranslated region and requires a minimal subset of translation initiation factors to occur, namely eukaryotic initiation factor (eIF) 2 and eIF3. Low-resolution structural information has revealed how the HCV IRES RNA binds human eIF3 and the 40S ribosomal subunit and positions the start codon for initiation. However, the exact nature of the interactions between the HCV IRES RNA and the translational machinery remains unknown. Using limited proteolysis and mass spectrometry, we show that distinct regions of human eIF3 are sufficient for binding to the HCV IRES RNA and the 40S subunit. Notably, the eIF3 subunit eIF3b is protected by HCV IRES RNA binding, yet is exposed in the complex when compared to subunits eIF3e, eIF3f, eIF3h, and eIF3l. Limited proteolysis reveals that eIF3 binding to the 40S ribosomal subunit occurs through many redundant interactions that can compensate for each other. These data suggest how the HCV IRES binds to specific regions of eIF3 to target the translational machinery to the viral genomic RNA and provide a framework for modeling the architecture of intact human eIF3.

Introduction

Human pathogens have evolved a wide variety of mechanisms to overcome cellular responses to infection. Hepatitis C virus (HCV), a positive-strand RNA virus that infects ~170 million people worldwide,[1] evades cellular responses to infection at the translation level by avoiding the need for most translation eukaryotic initiation factors (eIFs).[2] Instead of using mRNA cap-dependent eIFs (i.e., eIF4F and eIF4B), the HCV genomic RNA contains an internal ribosome entry site (IRES) that, together with translation initiation factors eIF2 and eIF3, directs the small (40S) ribosomal subunit to the correct start site to initiate the translation of the viral polyprotein (Fig. 1).[2] Human initiation factor eIF3, an 800-kDa complex of 13 subunits (eIF3a through eIF3m),[3] adopts a five-lobed structure that is thought to wrap around the platform of the 40S subunit and to serve as a scaffold for binding multiple translation initiation factors.[4,5] Initiation factor eIF2 is responsible for delivering initiator tRNA to the 40S subunit peptidyl tRNA site (P site) at the start of translation.[6]

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Abbreviations used: HCV, hepatitis C virus; IRES, internal ribosome entry site; eIF, eukaryotic initiation factor; P site, peptidyl tRNA site; cryo-EM, cryo electron microscopy; MS, mass spectrometry.

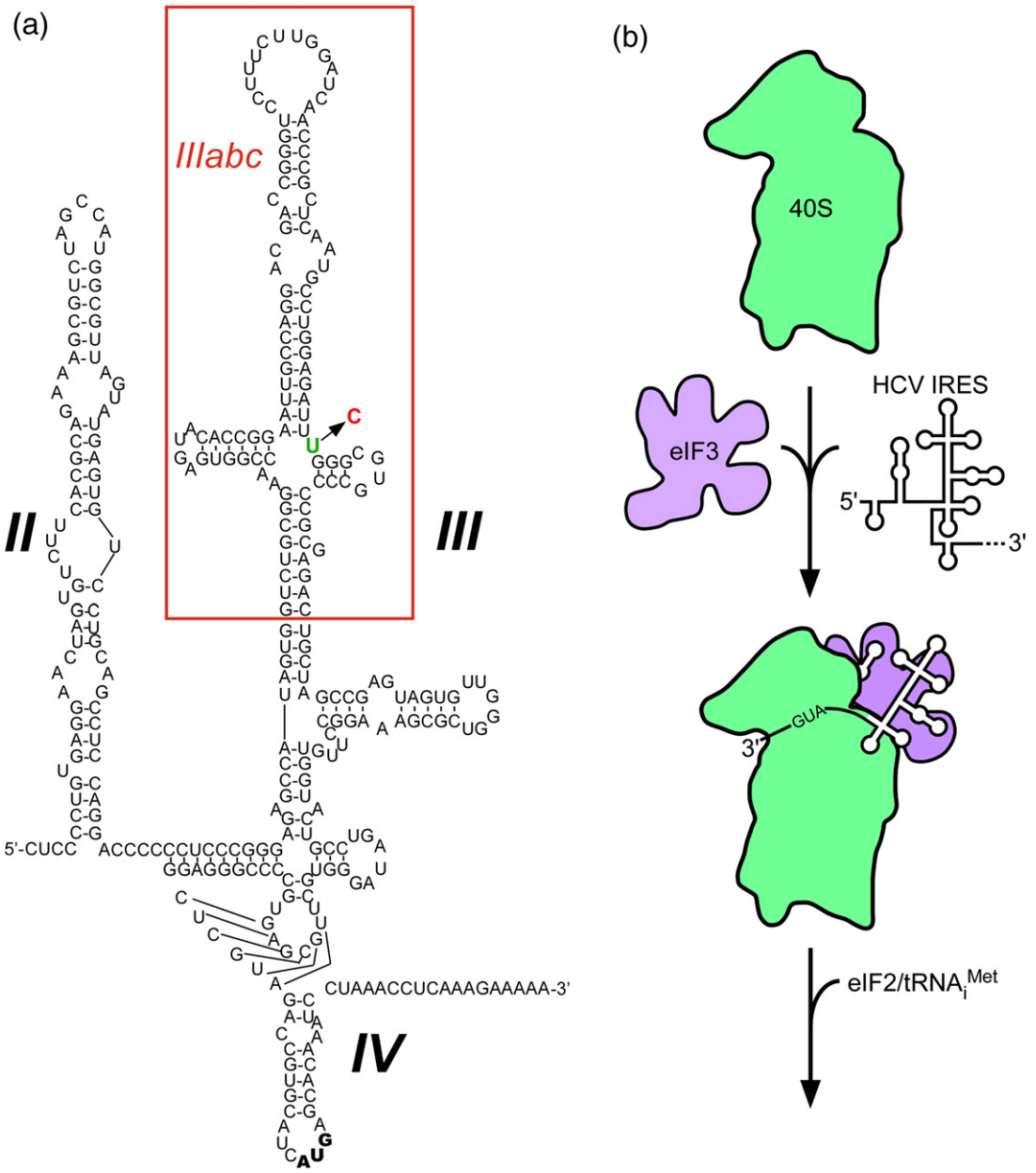

Fig. 1. Mechanism of HCV-IRES-mediated translation initiation. (a) Structure of domains II–IV of the HCV IRES element within the 5′ untranslated region of the HCV genomic RNA. Helical elements are numbered II–IV. Within the IRES, helices IIIa–IIIc (boxed) bind tightly to translation initiation factor eIF3. Nucleotide U228 (green) was mutated to C (red) to test the specificity of IRES interactions with eIF3. (b) IRES-mediated translation initiation requires only the HCV IRES, eIF3, the 40S subunit, and eIF2 *in vitro*.[2]

The HCV IRES element is a ~340-nucleotide RNA that folds into a complex tertiary structure that binds tightly to the human 40S subunit and to translation initiation factor eIF3 (Fig. 1b).[2] Interactions between the HCV IRES, the 40S subunit, and eIF3 lead to conformational changes in the HCV IRES/eIF3/40S subunit preinitiation complex that direct the start codon into the 40S subunit P site and stabilize initiator tRNA/eIF2 binding without the need for mRNA scanning (Fig. 1).[2,7,8] The structural basis for HCV-IRES-directed positioning of the genomic RNA start codon in the P site has been probed biochemically[9,10] and by cryo electron microscopy (cryo-EM) at low resolution.[11] Binding of the HCV IRES RNA to the platform region of the 40S subunit induces a conformational change in the 40S subunit that may be responsible for correct start codon positioning.[11] Biochemical