CRYSTAL STRUCTURE OF THE RNA-DEPENDENT RNA POLYMERASE OF HEPATITIS C VIRUS

Hepatitis C virus (HCV), an emerging virus, is becoming a major etiological agent of hepatocellular carcinoma. The World Health Organization has estimated that there are 170 million HCV carriers in the world with a substantial risk of developing liver cirrhosis and/or liver cancer [1]. There is currently neither a vaccine nor anti-HCV- specific therapeutics for HCV infection. Development of anti-HCV agents is thus a matter of much importance. HCV is a positive single-stranded RNA virus whose genome encodes one polyprotein, which is processed to structural and non-structural proteins by host and viral proteases. A non-structural protein at the polyprotein C-terminus, NS5B, includes the RNA-dependent RNA polymerase responsible for HCV replication. This RNA-dependent RNA polymerase is a major target for anti-HCV therapeutic development

We determined the first structure of the RNA-dependent RNA polymerase from HCV by X-ray crystallography using MIRAS/MAD phasing in a soluble form at beamline **BL45XU** [2] (Fig. 1). The overall fold of the catalytic domain of HCV RNA-dependent RNA polymerase is similar to other known polymerases: a right hand composed of fingers, palm and thumb domains. Beneath the webbed net, a wide solvent-accessible hole to the active site serves as the substrate entry path. The tight connection of the finger and thumb domains should restrain opening and closing motions during replication template binding. Corresponding domains in other polymerases are thought to grasp the template-primer duplex (Fig.2). The finger domain of HCV RNA-dependent RNA polymerase can be divided into two sub-domains. These two domains (αfinger and βfinger) are distinguished by their respective α-helix and

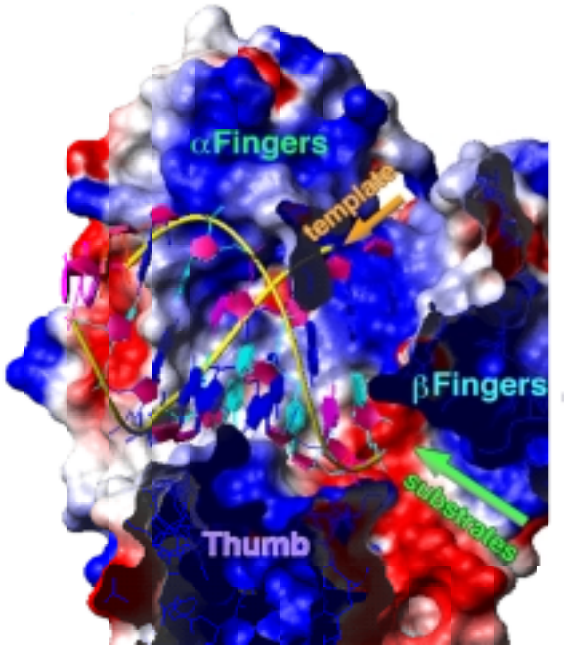

Fig. 2. Structural model of HCV RNA-dependent RNA polymerase ternary complex. The model was constructed based on the superimposition of the ternary complex of HIV reverse transcriptase [4] and HCV RNA-dependent RNA polymerase. The molecular surface is colored by blue to red corresponding to positive to negative electrostatic potential. The phosphodiester backbones of template and primer RNA are yellow. The green arrow indicates the entry path of substrate, and the brown arrow shows the direction of template movement.

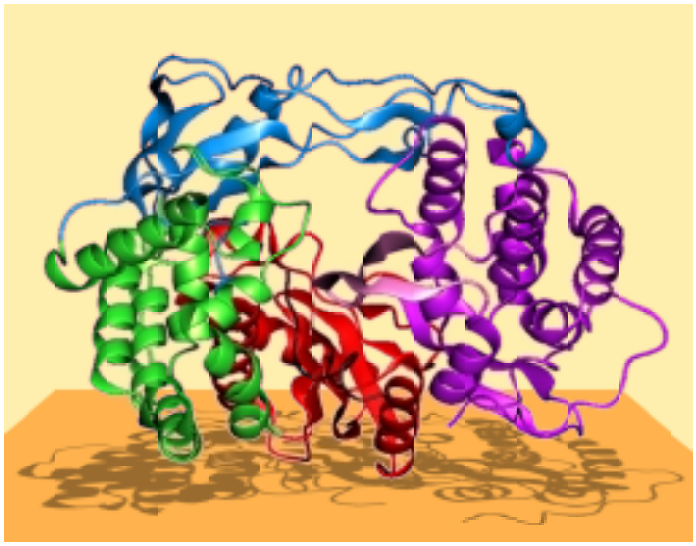

Fig. 1. Cartoon model of the RNA-dependent RNA polymerase of hepatitis-C virus. α fingers and βfingers subdomains, thumb, and palm domains are green, sky-blue, violet and red, respectively.

β-sheet dominance. These domains form a U-shaped valley which presumably serves as a binding or guiding site for the RNA template.

In HCV RNA-dependent RNA polymerase, the structure like a right-hand wearing a glove without any extra subdomains should contribute to its full *de novo* replication capability of both HCV genome and its anti-sense RNA without frequent abortive replication [3]. Furthermore, the structural model of the ternary complex illustrates a possible mechanism for RNA template and substrate selectivity (Fig. 3). This 3-D atomic structure will aid in the design of novel inhibitors of this polymerase, to be used as anti-HCV therapeutic agents.

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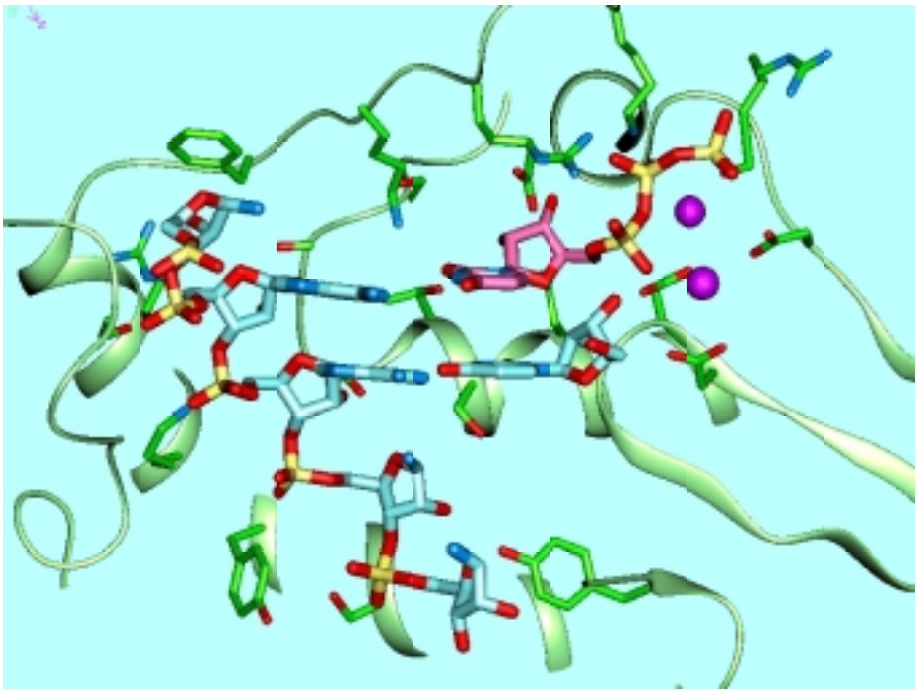

Fig. 3. Close-up view of HCV RNA-dependent RNA polymerase. The ternary model shows the proposed binding mode with the substrate, UTP, and the template RNA. The pink-, sky-blue- and green-colored carbons show the substrate, RNA duplex and HCV RNA-dependent RNA polymerase, respectively. Divalent cations are violet. The model was based on the complex structure with UTP and Mn^{2+}, and the superimposition with the ternary complex of HIV reverse transcriptase [4].