STRUCTURAL BIOLOGY

Kickstarting a viral RNA polymerase

Hepatitis C virus RNA–dependent RNA polymerase primes synthesis of its RNA genome with an inbuilt proteinaceous primer

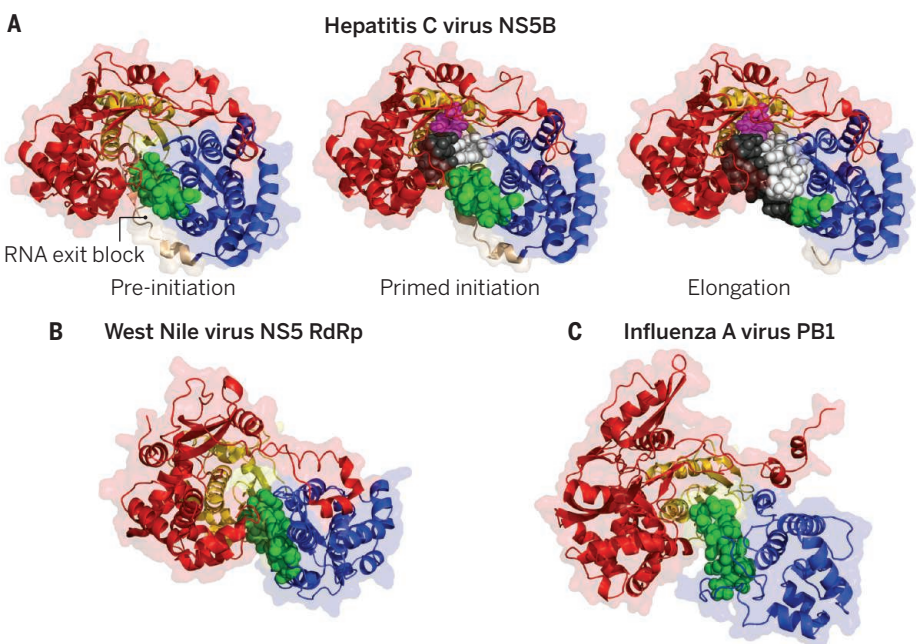
By **Stéphane Bressanelli**

RNA viruses are a ubiquitous class of pathogens that cause serious human, animal, and plant disease. To synthesize new viral genomes in infected hosts, RNA viruses encode special enzymes, RNA-dependent RNA polymerases (RdRps), which are thus prime targets for antiviral drug design. A case study is hepatitis C virus (HCV). HCV is a major human pathogen that chronically infects an estimated 170 million people worldwide, greatly increasing risk of developing life-threatening liver disease, including cirrhosis and cancer. New drugs against HCV have recently come to market that are extremely successful compared to the former standard of care and allow cure (that is, eradication of HCV from the patient) in over 95% of cases (*1*). Foremost among these new drugs is sofosbuvir, a nucleoside inhibitor targeting the active site of the HCV RdRp, NS5B. On page 771 of this issue, Appleby *et al.* describe new crystal structures of HCV NS5B (*2*) that are a major advance both in our basic understanding of RdRp activity and in the way sofosbuvir can inhibit HCV replication.

When they synthesize a new nucleic acid, most RNA (and DNA) polymerases do not truly "start" synthesis of the strand complementary to a template strand. Instead, they only elongate an existing RNA (or DNA) primer molecule. There is an exception, though: A ubiquitous class of viral RdRps can initiate RNA synthesis de novo, in the absence of an RNA primer. The conundrum presented by these enzymes is that during initiation, they must provide themselves with a proteinaceous "priming platform" to buttress the priming ribonucleotide that in effect acts as a one-nucleotide primer. This platform must then be removed to allow for subsequent elongation of the de novo synthesized short RNA, resulting in a situation equivalent to primer-driven nucleic acid synthesis found in other polymerases (*3*). A mechanism by which this problem has been solved was revealed by the x-ray crystal structure of the double-stranded RNA reovirus RdRp lambda3. In this case, the priming platform is a small, easily retracted protein loop, which allowed several rounds of RNA synthesis to be directly visualized (*4*).

In the case of HCV NS5B and other related RdRps, and unlike the case of reovirus lambda3, the exit path of the newly synthesized RNA is blocked by specific structural elements (in green and yellow in panel A of the figure, left) (*5*). Attempts to structurally characterize the mechanism by which the priming platform switches from its priming position to allow the transition to elongation of the RNA chain have long been unsuccessful. The main reason for this may seem paradoxical: In isolation, HCV NS5B is a self-inhibited RdRp with a low rate of initiation and a very poor transition to RNA chain elongation, a property clearly related to the block in the RNA exit path (*6*). This apparent "bug" in the system is actually a useful feature: In HCV-infected cells, a huge excess of NS5B is produced, almost all of which must be kept inactive.

How then did Appleby *et al.* catch HCV NS5B in the process of transitioning from initiation to elongation (panel A of the figure, middle)? The HCV community has produced an enormous amount of basic knowledge over 25 years of collaborative research from both academia and industry since the discovery of the main causative agent of "non-A, non-B hepatitis" (*7*). Appleby *et al.* cogently used this information and added several clever tricks of their own. Thus, they worked with an abnormal HCV strain, Japanese fulminant hepatitis 1 (JFH1), that caused a fulminant hepatitis instead of the usual slow course of HCV-related disease. This strain has extraordinary replicative capabilities (*8*) due to a much higher de novo RNA synthesis efficiency of its RdRp (*9*). In addition, they crafted an original combination of quasi-substrate and catalytic metal ion to lock JFH1 HCV NS5B in two successive steps of transition from initiation (panel A of the figure, middle and right). As usual with crystal structures, the new data provide a wealth of atomic-level



Priming the genome synthesis of an RNA virus. (**A**) Successive steps in RNA-dependent RNA polymerization by HCV NS5B. (**Left**) NS5B in its preinitiation conformation, with the RNA exit path blocked by the priming loop (in green) and carboxyl terminus (in yellow). (**Middle** and **right**) The new structures show how the primer loop recedes as NS5B opens, guiding the RNA along the opened path as successive nucleotides (in magenta) become incorporated in the growing RNA primer (in white) complementary to the template (in black). Among major pathogens, the flavivirus polymerase NS5 RdRp (from West Nile virus) (**B**) and the influenza virus RdRp catalytic subunit PB1 (**C**) also harbor an internal priming loop. For clarity, the PB1 carboxyl terminus is not displayed.

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details that in one go both confirm, extend, and revise previous knowledge about HCV NS5B and related RdRps.

The new structures definitively establish the HCV NS5B priming platform as the "β loop" (shown in green on panel A of the figure), an insertion in the "thumb" domain of viral RdRp. Such a priming loop, which had only been previously seen in HCV NS5B and its close RdRp relatives in the *Flaviviridae* family (*5*), has also recently been discovered in the influenza virus RdRp PB1 (*10*), extending the range of major pathogens to which Appleby *et al.*'s work is relevant. More importantly, Appleby *et al.* reveal the molecular mechanisms by which the β loop progressively recedes from the catalytic cleft of HCV NS5B, buttressing and guiding the nascent template-primer duplex along the RNA exit path right after initiation (panel A of the figure, middle).

Another major breakthrough is that, unlike in previous work of the same authors (*11*), the new structures are of so-called ternary complexes; that is, complexes with not only RNA but also the incoming, yet to-be-incorporated nucleotide at the catalytic site (in magenta on panel A of the figure, middle and right). The atomic details of incoming nucleotide recognition show unexpected features that explain how HCV NS5B may incorporate, and thus HCV replication be inhibited by, modified nucleosides such as sofosbuvir. As a final touch, the authors provide the structure of an actual ternary complex with incoming sofosbuvir.

Appleby et *al.*'s study is a technical tour de force providing much needed basic insights into viral RdRp structure and function, as well as the ways by which RdRp may be efficiently inhibited. The work was done with HCV NS5B (panel A of the figure), which was the first viral RdRp of known structure (*5*) and for which effective antiviral drugs now exist (*1*). But it also has far-reaching consequences for other major RNA viral pathogens for which new drugs are needed. These include West Nile virus (panel B of the figure) and Dengue virus in the same *Flaviviridae* family as HCV (*5*) but also more distantly related RNA viruses such as influenza viruses (panel C of the figure) (*10*). ■

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Breaking the intestinal barrier to deliver drugs

The structure of a tight junction protein bound to a disruptive toxin may guide drug delivery strategies

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Many drugs must be absorbed into the circulation for medicinal effects to occur at the intended sites of action, and so a holy grail of drug delivery is to improve the passage of pharmaceuticals across tissue barriers. Most oral drugs are absorbed in the small intestine, where the lumen is lined with epithelial cells. Thus, new therapeutic strategies for efficient oral delivery can benefit from a better understanding of the protein complexes, such as the tight junction, that maintain the integrity of this epithelium. On page 775 of this issue, Saitoh *et al.* (*1*) report the structure of a tight junction constituent called claudin-19, bound to a bacterial toxin called *Clostridium perfringens* enterotoxin (CPE), an agent that disrupts tight junctions and is a major cause of foodborne illness by this pathogen. The structural information may be useful in developing specific claudin-targeted compounds that improve drug delivery across tissue barriers that currently limit drug absorption.

Tight junctions connect adjacent endothelial cells and control diffusion of solutes between them. In the skin, lungs, eyes, and gut, these junctions form epithelial barriers against harmful agents in the environment, and in brain capillaries they form the blood-brain barrier that protects the brain against leakage of toxic agents from the blood. Skin and blood-brain barrier tight junctions are largely impermeable, reflecting their essential barrier function in these tissues, whereas in the intestine and other epithelia that transport molecules, they display selective permeability for ions such as sodium, calcium, and magnesium (*2*). There is ample evidence that claudins—a 27-member family of transmembrane proteins—are responsible for the "paracellular barrier" functions in epithelia (*3*).

The claudins, which are expressed in tissue-specific combinations, form the extracellular compartment of the junctional complex together with other transmembrane barrier proteins such as occludin. Within an epithelial cell, claudins are linked to peripheral scaffolding proteins (e.g., ZO-1), that in turn are linked to the cytoskeleton (actin and microtubules) through linker proteins (see the figure). Several of these intercellular proteins have phosphorylation sites that control assembly of the tight junction complex and hence the paracellular barrier. Tight junctions are dynamic structures in that they are sensitive to changes in the local environment—for example, those caused by proinflammatory cytokines and bacterial pathogens (*2*). Knowledge of how these junctions are regulated and affected by toxins such as CPE is essential for understanding tissue homeostasis and for tight junction–targeted drug development.

> ***"...structural information may be useful in developing specific claudin-targeted compounds that improve drug delivery..."***

The paracellular barrier function of tight junctions is attributed to several claudins, including the CPE-binding claudin-3, -4, and -19. However, a paracellular channel function has been suggested for others, including claudin-2 and -15. Disordering of the claudin structure may open either or both of two distinct channel populations of the tight junction: the pore pathway that routes small ions, and the leak pathway that allows the passage of drugs and macromolecules. In mice, ion pores in intestinal tight junctions provide the passage of sodium ions into the intestinal lumen, which is necessary for nutrient absorption (*4*). For drug delivery, claudin-targeted agents must open the leak pathway in a controlled, reversible manner that does not disturb ion transport and tissue homeostasis.

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