Complexes of Hepatitis C virus RNA polymerase: insights into nucleotide import and *de novo* priming.

Rachel Trowbridge, Damien O'Farrell, Henric Ekstrand, Dave Rowlands and Joachim Jäger

Introduction

Hepatitis C virus (HCV) infection is found throughout the world with prevalences in blood donors ranging from 0.4% (US/U.K.) to more than 14% (Egypt). Random screening of blood donor populations has indicated that there could be close to 500 million chronic carriers of the virus, which highlights HCV as a major human pathogen. The virus is transmitted primarily by the parenteral route, and many HCV-infected individuals are intravenous drug users or recipients of blood products. Although with serological monitoring of blood for anti-HCV antibodies the incidence of transfusion associated hepatitis has decreased dramatically.

So far, treatment of chronic HCV infections is limited to interferon-α therapy which is successful in only 40% of treated patients. After cessation of therapy, about 70% of these responders relapse and only 25% of patients show a long term prognosis. The response can possibly be improved using combination therapy with ribavirin. Even in this case, 60% of patients do not show a long-term response, substantiating the need for a more effective therapy against chronic hepatitis C. Therefore, the RNA dependent RNA polymerase of HCV has been selected as a key target for developing specific antiviral therapy.

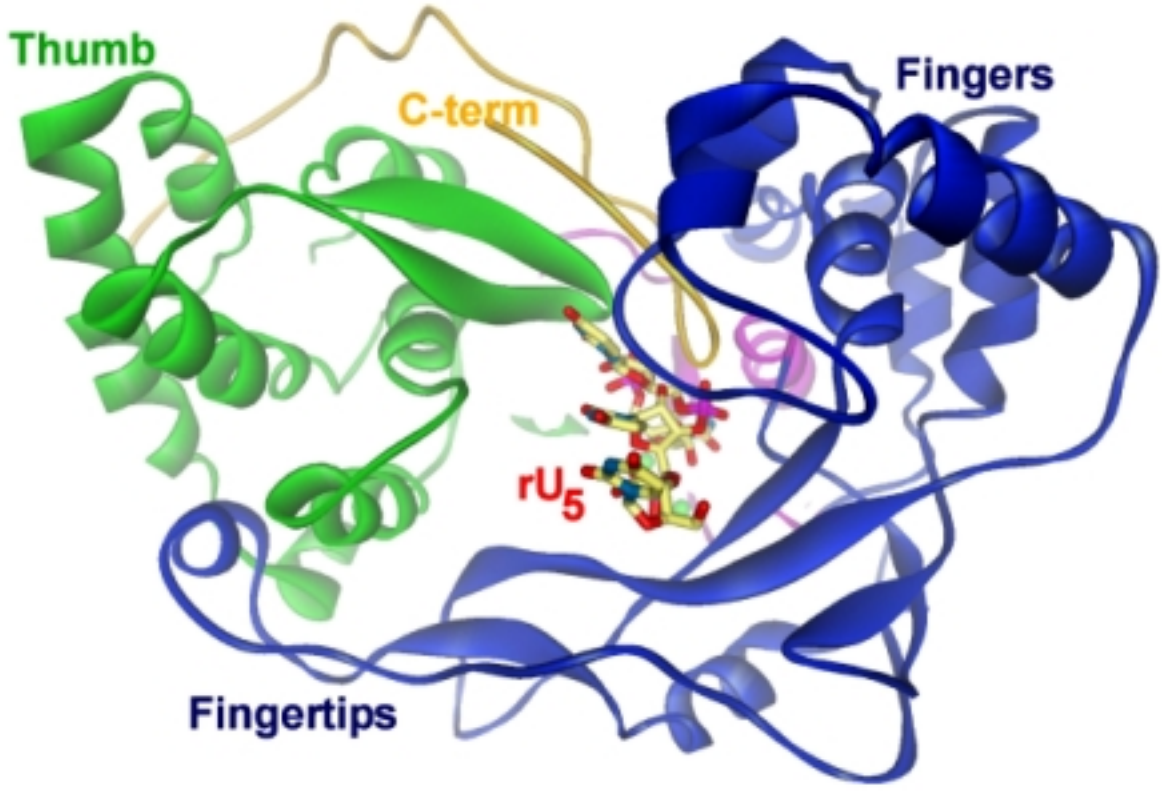

Figure: Ribbon diagram of HCV RNA polymerase (NS5B) complexed with a short RNA oligomer soaked into the template binding site.

Structural studies

Wild-type and mutant HCV polymerase (J4 derived NS5b; M_r 66,000) is bacterially expressed, yielding about 20mg of pure HC-J4 NS5B per litre LB medium. Purification is based on affinity chromatography using Ni-NTA superflow resin followed by a second step using poly(U) sepharose. The protein is fully active and crystals can be grown within four days to two weeks.

The native structure of HC-J4 polymerase has been refined against data extending to 2.00Å resolution. The polymerase folds into the characteristic fingers, palm and thumb subdomains. The "fingertips" are in close contact with the thumb subdomain. During the catalytic cycle it is plausible that the polymerase maintains the closed conformation similar to that seen in the native crystal structure.

Several complexes have now been established by crystal soaking using short RNAs, $r(U)_5$, and with various NTPs. These complexes reveal the binding sites of the viral RNA template (see Figure) and the incoming nucleotide-triphosphate. Using HIV-1 RT as a reference, modeling of dsRNA primer-template into the NS5b polymerase active site reveals that the thumb subdomain has to be displaced, possibly indicating how the polymerase translocates on the viral genome.

Structural and functional studies on several site-directed mutants of HC-J4 NS5B are currently underway. The mutants have been selected to probe the functional role of certain active site residues in controlling processivity and fidelity of HCV RNA polymerase.

Structure-based drug design and high-throughput screening

Using the programs SPOCK (J Christopher, Texas A&M) and SPROUT (AP Johnson, Leeds), several small cavities in the vicinity of the active site have been characterized with regard to possible binding sites for highly specific antivirals. Four suitable compounds have been short-listed for further structural studies, two of which have shown sub-millimolar IC50s. The binding studies are paralleled by a mutational analysis of the putative drug binding sites.

In parallel, several drug screening campaigns are underway using a non-radioactive, antibody-based platform technology developed by our spin-out company RepliZyme Ltd.

References

Jäger, J. & Pata, J. (1999) Getting A Grip: Polymerase and their substrate complexes. *Curr. Opin. Struct. Biol*. **9**, 21–28

Jäger, J. & Pata, J. (2002) Viral RNA polymerases in “Structure Function Relationships of Human Pathogenic Viruses” p53-84. Kluwer Academic/Plenum Publishers, New York, London.

O’Farrell, D., Trowbridge, R., Stock, N., Rowlands, D. & Jäger, J. (2002) Co-crystal structures of Hepatitis C virus RNA polymerase (HC-J4): structural evidence for nucleotide import and *de novo* initiation (submitted).

Collaborators
Tobias Restle, MPI Dortmund, Germany
Karla Kirkegaard, Stanford, USA
Seishi Murakami, Kanazawa, Japan
Jens Bukh, NIH, Bethesda, USA
Berwyn Clarke, VisGen Ltd., Cambridge

Funding
We gratefully acknowledge financial support from Action Research, BBSRC (Microsciences Ltd), RepliZyme Ltd and GSK UK.