Structural and functional studies on Hepatitis C virus non-structural proteins.

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Hepatitis C virus (HCV) is an increasingly important cause of liver disease. The virus has a single stranded positive sense RNA genome of 9.5kb that contains a long open reading frame encoding a single polyprotein of 3000 amino acids. This is cleaved into 10 individual polypeptides by a combination of host cell and virus specific proteases (see Figure 1). The molecular mechanisms of pathogenesis remain to be elucidated. To this end my laboratory is interested in the potential for the non-structural proteins (expressed from the 3' end of the genome and designated non-structural as they do not form part of the viral particle) to interfere with host cell metabolism and signal transduction pathways.

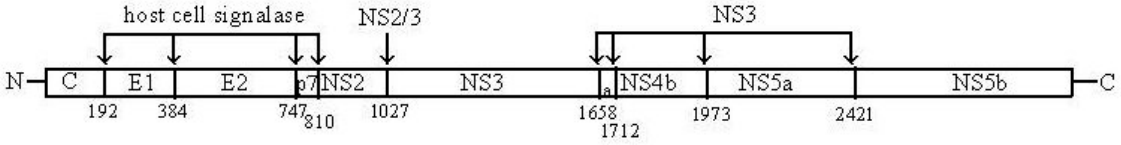

Fig. 1 Polyprotein cleavage strategy of HCV

The non-structural protein NS3 has three enzymatic activities: a proteinase and a helicase/NTPase. Recently, catalytically inactive NS3 fragments containing an arginine-rich motif have been reported to interact with, and inhibit, the catalytic subunit of cAMP-dependent protein kinase (PKA C-subunit). We have used recombinant baculoviruses to express full-length, catalytically-active NS3 and have shown that it is also able to inhibit PKA C-subunit *in vitro*. However both mutational analysis and experiments in which a constant ATP concentration was maintained by the addition of an ATP regeneration system demonstrated that the ability to inhibit PKA was due to ATPase activity. We are currently pursuing the functional consequences of NS3 expression in mammalian cell lines to determine whether ATPase activity might play a role in pathogenesis of this virus.

The other non-structural protein that is a focus for the laboratory is NS5A. We have shown that two closely spaced poly-proline motifs in NS5A interact with the SH3 domains of members of the Src family of protein tyrosine kinases. We are currently using phage display, surface plasmon resonance and *in vitro* and *in vivo* binding assays to understand more precisely these interactions. Additionally we are expressing NS5A both stably and transiently in mammalian cells to identify the functional consequences of these interactions, in particular we are using the newly developed BacMAM system - baculovirus vectors with mammalian specific promoters that are able efficiently to enter mammalian cells and drive expression of foreign genes. Preliminary data suggest that NS5A perturbs the activation of the AP1 transcription factor - implying a role for perturbation of the MAP kinase pathway.

Publications:

Aoubala, M., Holt, J., Clegg, R.A., Rowlands, D.J. & Harris, M. (2001) The inhibition of cAMP-dependent protein kinase by full length hepatitis C virus NS3/4A complex is due to ATP hydrolysis. *Journal of General Virology* **82**, 1637-1646.

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Funding: BBSRC, MRC.